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CHARLES EDWARD FAIRMAN

1856–1934

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(WITH PORTRAIT)

As the Mycological Society of America, in session at Pittsburgh on December 27, was engaged in drafting a formal expression of regret at the loss by death of several of its number during 1934, Charles Edward Fairman, a charter member and one of the oldest individuals in the organization lay dying at his home in Lyndonville, New York. In his passing American mycology loses one of the few students of its Pyrenomycetes possessed of a comprehensive knowledge of species. He was a mycologist of the old school, to whom the study of fungi was merely an avocation. By profession a country physician, he spent practically all the years of his long and busy life in the little hamlet in which he died, remote from mycological centers and without the stimulus of contact with fellow mycologists. His death came suddenly and wholly unexpectedly the day before his seventy-eighth birthday.

Doctor Fairman was born December 28, 1856, in the village of Yates, near the southern shore of Lake Ontario in Orleans County about midway between Rochester and Buffalo. His later home at Lyndonville lies only a few miles to the south. Both of his parents were teachers. From them he must have inherited his love for study and gift for research. During his boyhood his father taught at Yates and Medina, but in 1868

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accepted a call to Shurtleff College at Alton, Illinois, where for five years he was professor of mathematics. In 1873, which was his son's senior year in college, he returned to New York to become principal of Cook Academy at Havana, now Montour Falls. This move resulted in his son leaving Shurtleff before graduation, to enter the University of Rochester. He graduated there in June 1874, when only seventeen years and six months of age. He then entered the St. Louis Medical School, and in 1877 received his M.D. The same year the University of Rochester conferred on him their M.A. Before his twenty-first birthday he was back in the home of his childhood established in the practice of medicine at Lyndonville. He had been an extremely brilliant student, and he was to be outstandingly successful in his profession. As the years passed he became the highly esteemed and much loved family doctor of the whole countryside. In July 1927 the Orleans County Medical Society tendered him a testimonial dinner in celebration of his fiftieth anniversary in the practice of medicine. He gave seven additional years of continuous service before his death.

Doctor F. W. Scott of Medina, a lifelong associate and friend, spoke at the dinner, and in response to my request wrote me as follows concerning the attainments of Doctor Fairman in the field of medicine.

"It is a compliment to be privileged to submit my version of Doctor Fairman's status as a physician. As you suggest, I am aware of his contributions and achievements in research on the fungi, the consideration of which is without my province and with which you are very much more familiar than I am. I also know that this mycological study has been and always will be held in subserviency, and that elevated position and emoluments, financial considerations and freedom from laborious life, have all been renounced that he might remain engaged in his chosen life work, the practice of general medicine. Though he was destined by reason of natural endowments, scholarly attainments, moral excellence and general acumen to become much better than mediocre in any endeavor in which fate might direct him, he never aspired to a specialty in medicine. In fact he deprecated any suggestion or thought of it. For fifty consecutive, continu-

ous years he has and still continues to offer his ministrations to the sick. A significant record of its kind I believe! He is an internist, pathologist, and diagnostician whose worth is recognized beyond the confines of his usual field of labor, and is reflected in the attitude of his loyal, loving clientele and confrères. This then is an estimate of Doctor Fairman; conservative, fair, universal, and not to be confused with posthumous eulogy."

Doctor Fairman was about thirty years of age when he first began to study the fungi. His interest was aroused when he and his father-in-law, Doctor John D. Warren of Lyndonville, undertook to raise mushrooms. He came then into possession of a book on mycology in which he met the sentence: "A wide field is open for research in this branch of botany." The statement spurred his ambition, and led finally to his undertaking serious investigations in the field of the sphaeriaceous fungi. He made contact through correspondence with Ellis, Peck, Saccardo, Rehm, Arthur, and other students in America and abroad, and slowly but surely built up a personal mycological library, and accumulated an extensive herbarium. He obtained most of the general taxonomic works, including Saccardo's Sylloge Fungorum; and his herbarium contains various exsiccati which were invaluable to him in his comparative studies. A set of Rehm's Ascomyceten was purchased jointly with Elias J. Durand, one taking the Pyrenomycetes, the other the Discomycetes. His contact with Ellis was most fruitful, and numerous species collected by him near Lyndonville, were distributed by Ellis in North American Fungi and Fungi Columbiani. He described a considerable number of new species and erected a few new genera. His taxonomic contributions were chiefly on the Pyrenomycetes and Fungi Imperfecti. In his later years he specialized somewhat on the Lophiostomataceae, but though his mycological friends urged him to prepare a monographic treatise on this family it was never accomplished. A quotation from one of his letters to the writer is interesting in this connection. He said:

"I do not consider the miscellaneous naming of fungi for other people as a high type of mycological work. When one attempts it he puts himself in the position of describing new things in

groups which need revision, only to find a little later his ground genera disenthroned and the species widely scattered. My reason for writing my paper on 'the fungi of our common nuts and pits' was that I had material on these things which needed to be brought to light. Lloyd complained and so has Doctor Thaxter that I have written so little of a monographic nature, but you know that this type of publication requires access to things a country doctor has not and visits to herbaria and libraries for which he has no time. I wish you to know that I do not look with favor upon any but accurate scientific work, and that opportunity has denied me what I most desire. 'I ne'er shall see fair Carcassonne.' "

Dr. Fairman's modest nature was his outstanding charm. Though he speaks thus disparagingly of his miscellaneous naming of Pyrenomycetes for others, it is well known that few students have been able and willing to attempt identifications throughout this large and puzzling group, though the need for it has been great. In other letters he says:

"I have not been able to complete my work on *Lophiostoma* for lack of time. I thought when I reached my present age no one would wish me for a physician, but it is my lot to have more to do. I put in all my spare time in mycological work. I will not yield to any one in devotion, for I often get up at eleven to one o'clock at night after an hour or two in bed and put in some time on the fungi. I can truly say I have used all available time and strength.

"My publications cannot be in the nature of monographs. I enjoy being a free lance in the field, and while I may run up against Quixotian windmills, sally forth every day to the fray.

"If I have a little time in the morning as I start on my calls I stop in the woods and search for fungi. If I find an interesting specimen I take it with me, and when I reach home for lunch I get out my microscope and study it. If it proves worth while I give it further study that evening, and get it ready for my files."

In November 1918 he wrote:

"I have been engaged in fighting the influenza epidemic. In addition to my private practice I have to attend the duties of the local health office. I have had to see as high as thirty-five

patients a day, and in country practice that means work. I have worked from early morning until 11 or 12 at night and sometimes all night. Therefore, I have not been able to look at the specimens you sent. Though the influenza is now abating I am physically and mentally unequal to the critical study of fungi. I am anxious to again have leisure for my hobby."

Other mycologists, realizing that the life of a country physician is a most strenuous one, have doubtless wondered how Doctor Fairman was able to find time and energy to pursue diligently a scientific study in addition to his time-consuming and exhausting professional duties. These excerpts from his letters reveal that it was only possible because he had a tenacity of purpose and a depth of interest in his mycological endeavors which many others do not possess.

Doctor Fairman was not personally known to a wide circle of American students of the fungi. He travelled little, and only rarely attended botanical meetings. When the occasion offered, it was his custom to make short visits to nearby mycological centers for study amid library and herbarium facilities superior to his own. Perhaps less frequently other mycologists visited him at Lyndonville. Those with whom he thus made contact found him a courteous host and an interesting and charming companion. He was a quiet spoken, unusually unassuming man, of something less than medium height and of somewhat stocky build. The accompanying photograph, taken in a photographer's studio at Medina, shows him at about the age of sixty. It was published by C. G. Lloyd on the cover of Mycological Notes No. 60, in 1919, accompanied by a brief statement of appreciation. It is unfortunate that the picture does not show him in his own study surrounded by his books and specimens. The microscope pictured, though old fashioned, was optically excellent, and was supplemented by a thoroughly modern binocular. In other respects he had adequately equipped himself to do accurate work. In some way, probably through his hospital contacts, he had found it possible to imbed his material in paraffin and obtain stained sections when they were necessary.

Some years ago he made a will bequeathing his herbarium and mycological library, including his correspondence of mycological

interest, to his alma mater, the University of Rochester. He was especially desirous that his accumulations should be preserved as an entity. Recent correspondence indicates that his wishes are to be met. As his herbarium contains the type specimens of all of his own species its careful preservation is of course highly desirable.

Doctor Fairman was a member of the Rochester Academy of Science, and a number of his papers were published in its Proceedings. Other contributions appeared in Annales Mycologici, the Journal of Mycology, and Mycologia. In 1920 he published privately at Lyndonville a little pamphlet on the ascomycetous fungi which he had isolated from human excreta. Perhaps his paper of most general interest is that dealing with the fungi occurring on nuts and pits. The following list of his mycological publications is believed to be essentially complete.

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PARASITISM OF DISPIRA CORNUTA [1]

THEODORE T. AYERS [2]

(WITH 4 TEXT FIGURES)

INTRODUCTION

The early studies on *Dispira cornuta* van Tiegh., although few in number and limited in scope, showed clearly that the fungus is one of considerable mycologic interest in its structure, reproduction and development and in its relationship and taxonomic position. Of even greater interest is its parasitism, for this fungus has been reported as an obligate parasite on members of the Mucorales, the order to which it, itself, apparently belongs, even though the early investigators using gross cultures only succeeded in demonstrating experimentally that it would parasitize a few unidentified species of *Mucor*. It would seem desirable to determine whether *Dispira cornuta* is parasitic only on species of *Mucor*, or on members of other genera within the Mucorales, on other Phycomycetes, or even on representatives of the other main groups of fungi. Yet any investigation of the parasitism of this fungus necessitates securing it in pure culture, hence, when, as previously reported (**2**) the writer found that *Dispira cornuta* was not an obligate parasite but could be grown successfully in pure culture on artificial media rich in proteins, a study of this problem was undertaken. In addition certain details of the life history of the fungus both as a saprophyte and as a parasite were examined. The results of these investigations are presented in the following paper.

HISTORY AND TAXONOMY

Dispira cornuta has been collected rarely since it was reported originally from France (1875) by van Tieghem (**21**), who found

[1] Contribution from the Laboratories of Cryptogamic Botany, Harvard University, No. 108.

[2] At this time, the writer wishes to express his appreciation of helpful criticism, advice, and encouragement to Dr. Wm. H. Weston, Jr., who suggested this investigation.

it growing associated with various species of the Mucorales on rat dung. Since the sporophores of the fungus branched at their tips dichotomously into two equal divisions and the resultant branches bore horn-like growths at the base of the fertile heads, he gave it the appropriate name of *Dispira cornuta*. From North America on rat dung which had been collected in Ohio, a fungus resembling *D. cornuta* was described by Thaxter (**18**) in 1895. In this American fungus, however, Thaxter observed that the sporophores branched with a false dichotomy; several spore chains were arranged in whorls around the tips of the primary and secondary sterigmata, and furthermore, each spore chain was composed of two spores. In contrast, van Tieghem has described the sporophores of *D. cornuta* as dividing dichotomously at their tips while a single spore chain composed of six spores was attached to the apex of each terminal sterigma. Because of these differences Thaxter concluded that the American fungus was a new species of *Dispira*, and named it *D. americana*. This fungus was considered to be a distinct species until 1906 when Bainier (**3**) called attention to the errors in van Tieghem's illustrations of *D. cornuta*, and showed that the spore chains in reality comprised two spores each instead of the six spores illustrated, and in reality were arranged in whorls around the tips of the primary and secondary sterigmata. Also Bainier illustrated *D. cornuta* as branching pseudodichotomously. Since these two fungi were so similar in morphological characters, Bainier decided that *D. cornuta* and *D. americana* were synonyms or the same fungus and retained the binomial, *Dispira cornuta* because of its priority. More recently a fungus resembling *D. cornuta* was reported from England (1926) by Elliott (**9**) as *D. circinata*. According to her, this fungus differed from *D. cornuta* in the following respects: "in having five or six instead of two branches radiating out from the erect conidiophore. The branches bearing the *Aspergillus*-like heads of conidia have the same peculiar method of growth, but in *D. cornuta* the branch which is equivalent in origin to the encircling branch of *D. circinata* is shorter and projects outward in a horn-like fashion. Further in *D. cornuta* each of the globular heads bears only one chain of spores." It should be noted that this description fits

van Tieghem's original and faulty delineation of the fungus rather than Bainier's emendation. However, these variations from the description of *D. cornuta* and the identity of *D. circinata* will be considered in more detail later.

In the fall of 1927, a fungus possessing the essential characteristics of *D. cornuta* was isolated by the writer (**2**) from growth with an unidentified species of *Mucor* on hog dung which had been collected at Waltham, Mass. Since this first collection, the same fungus has been collected repeatedly and further study has shown that without doubt it is *Dispira cornuta*. In corroboration it should be noted that Dr. Thaxter examined the fungus and expressed the opinion that it was identical with his *D. americana* and the *D. cornuta* of van Tieghem. The number of collections of this fungus made during the investigation, the dates of collection, the different substrata upon which the fungus and its host grew, are summarized in Table I.

TABLE I

SOURCE OF COLLECTIONS

Place of Collection	Date of Collection	Substratum	Host
Station No. 1, Waltham, Mass...	Dec. 8, 1927	Hog dung	*Mucor* sp.
Station No. 2, Cambridge, Mass.	May 7, 1928	Dog "	" "
Station No. 1, Waltham, Mass...	May 27, 1928	Hog "	" "
Station No. 3, Waltham, Mass...	Dec. 8, 1928	Hog "	" "
Station No. 1, Waltham, Mass...	Dec. 7, 1928	Hog "	" "
Station No. 4, Cambridge, Mass.	Dec. 10, 1928	Rat "	" "

Considering the number of collections listed in Table I, it is obvious that *D. cornuta* is more common than hitherto has been supposed. The rarity of this fungus formerly might have been due either to its minute size, its fairly brief appearance in gross culture, or its limitation in nature to those members of the Mucorales which inhabit substrata of high nitrogen content such as dung of rat, hog, or dog. That the fungus was collected repeatedly from the same source without difficulty and at intervals of six months or more seems to indicate that it is fairly persistent in certain localities.

From these collections, isolations of *D. cornuta* and *Mucor* sp. were made, the *Dispira*, even though always considered an obli-

gate parasite, being successfully cultured on nutrient media without a host by methods already described by the writer (2) in a previous paper. After it was learned that *D. cornuta* could be cultivated on certain media in the absence of a mucoraceous host, single spore isolations by the dilution method were made for the purpose of determining if there were different strains among these collections. Even though they were secured from various sources at different times, comparison of the several

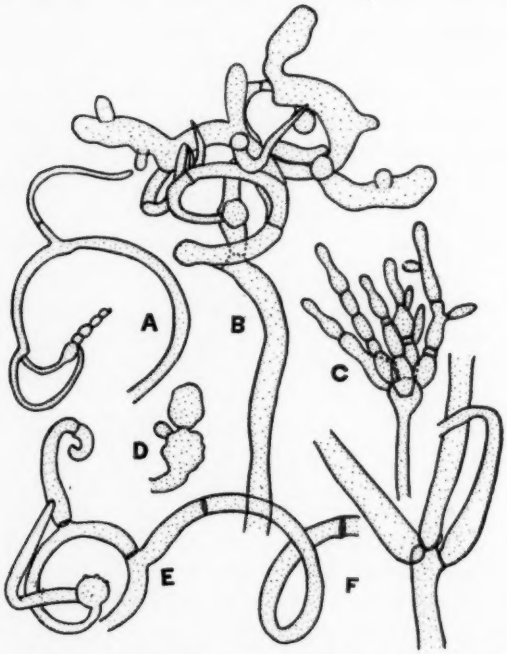

FIG. 1. A, C, D, aberrant forms of fertile heads, sterigmata (A, C 1000 ×, D 1700 ×); B, young fertile hypha showing proliferation and modification of various parts. 1000 ×; E, fertile hypha branching at tip and forming two branches of unequal length. 1000 ×; F, fertile hyphae producing three branches instead of the usual number of two. 1000 ×.

single spore isolations on the same media under identical conditions failed to show any physiological or morphological differences. It was observed, however, that these isolations were

very plastic in culture, when grown either alone or with a host. For example, under different external conditions such as moisture and humidity, the fertile hyphae (sporangiophores) not only varied in height and produced 2, 3, 4 or more branches (FIG. 1, *f*) of varying length (FIG. 1, *e*), but also branched both dichotomously and pseudodichotomously at their tips. Likewise the fertile heads on these branches (FIG. 1, *c*, *d*, *e*) and the horns at the base of these heads (FIG. 1, *b*, *e*) proliferated considerably and assumed aberrant forms instead of the shapes which are so characteristic of this fungus. Even the sterigmata (FIG. 1, *a*, *b*, *d*, *e*) in some instances became abnormal and resembled van Tieghem's illustrations of a spore chain which showed six spores instead of two for each chain. Nevertheless under no conditions during this investigation were more than two spores in each chain observed. Inasmuch as conditions of culture may induce such decided variations in the branching of the sporophores and the structure of the sporiferous heads it seems clear that the characteristics of these parts, although they have been used for specific identifications are not sufficiently dependable. After becoming familiar with these variations and comparing them with the different descriptions and illustrations given for *D. cornuta*, *D. americana*, and *D. circinata*, it is realized that these three names are synonyms and apply to the same fungus, hence because of its priority, *D. cornuta* should be used.

MORPHOLOGY

The several single spore isolations from the various collections of the fungus were not only studied comparatively to see whether they comprised different strains but also were mated on different media either alone or on suitable hosts to determine whether different sexes were present and could be induced to develop a sexual stage. As yet the fungus has never been found to develop any sexual stage whatever. To be sure, Thaxter (**18**) described the sexual spores or zygospores in 1895 but later he reported (**4**) that it was the parasitic cells of *Parasitella* on *Mucor* sp. which he had interpreted as zygospores. Despite the fact that hundreds of crosses have been made, using different isolations and subjecting them to various external conditions, no sexual stage has

ever appeared in these cultures. But bodies suggestive of zygospores or azygospores have been found in various cultures of *D. cornuta* (FIG. 2, *c–j* incl.). These cells which develop freely even under the usual conditions of cultivation, resemble somewhat the zygospores reported for *Blakeslea*, *Choanephora* and *Syncephalis* in their thick walls and large oil globules, but so far the essential stages of conjugation, which occur in the formation of true zygospores, have never been observed. At this time, no definite function can be assigned to them with certainty although it seems probable that they enable the fungus to pass through periods unfavorable for growth and sporulation. Besides these bodies just described, chlamydospores (FIG. 2, *a*, *b*) are formed by this fungus when it is planted alone on certain media such as malt agar. Their formation probably accounts for the fact that *D. cornuta*, although reduced to a meagre growth of mycelium without sporophores may retain its viability until the agar medium is almost completely desiccated as reported previously.

RANGE OF PARASITISM

After securing *Dispira* in pure culture on artificial media it was possible to attack effectively one of the most interesting problems presented by this organism, namely its parasitism.

As pointed out in a previous paper (**2**) van Tieghem had attempted to culture *D. cornuta* alone on artificial media but had found that although the spores enlarged and produced germ tubes they then remained quiescent. Since, however, he was able to grow the fungus in the presence of various species of *Mucor* he concluded that it was an obligate parasite on members of the Mucoraceae. In the present investigation, therefore, it seemed desirable to determine whether the fungus were parasitic on species of *Mucor* only or could attack other members of the Mucorales and even entirely unrelated fungi of other groups. Accordingly several representative members of the Myxomycetes, Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti were tested as possible hosts for this parasite. To determine whether these various fungi were hosts for *D. cornuta*, pure cultures of these suspected hosts were transferred to tubes of various solid media, usually potato-rat dung-agar, which had

been found to be suitable for the growth of these fungi but not for the growth and sporulation of *D. cornuta*. These cultures were then inoculated with material from pure cultures of *D. cornuta* derived from either single spores or spores and fragments of the fertile hyphae. To make certain that *D. cornuta* had not become adapted to the media used in these tests, a duplicate series of pure cultures of the parasite was transferred to the media used. The cultures of the suspected host inoculated with the parasite and the duplicate series of the parasite alone, were stored in the laboratory under conditions which were suitable for the growth of both fungi, and frequent observations were made on the subsequent growth of these organisms until it was certain that *D. cornuta* was either parasitic or not parasitic on the fungus used in the test.

In Table II are given the fungi tested as hosts, together with the extent of the growth and of the sporulation of *D. cornuta* on those which proved to be susceptible, the extent of growth and the sporulation being described by such terms as trace, meagre, good, etc., and these terms are expressed by symbols in the table as explained in the footnotes. Since this study is not quantitative in nature, these terms suffice to convey to the reader a relative idea of the growth and sporulation of *D. cornuta* on its different hosts. Examination of this table discloses the following points of interest:

Dispira cornuta was found to be parasitic on members of the Mucorales only, although as shown in the above table, it was tested on an adequate number of fungi from different groups including representatives from the Myxomycetes (Acrasiales), Phycomycetes (Mucorales, Entomophthoraceae, Saprolegniaceae, and Pythiaceae) Ascomycetes (Endomycetaceae, Phacidiaceae, Dermataceae, and Sphaeriaceae) Basidiomycetes (Ustilaginaceae, Tilletiaceae, Thelephoraceae, Hydnaceae, Polyporaceae, and Agaricaceae), and Fungi Imperfecti (Moniliaceae, Dematiaceae, Phomaceae, and Melanconiaceae).

Within the Mucorales, *D. cornuta* has been found to be parasitic, not only on species of *Mucor*, but on representatives from every known family of this order. These hosts, grouped according to a modification of Lendner's key (14) are as follows:

TABLE II

HOST RANGE OF *Dispira cornuta*

Fungi[a] Tested as Hosts	Growth of *D. cornuta*		Fungi[a] Tested as Hosts	Growth of *D. cornuta*	
	Vegetative	Sporulation		Vegetative	Sporulation
Myxomycetes (Acrasiales)			Ascomycetes		
Dictyostelium mucoroides Bref.	—[b]	—	*Endomyces capsulatus* Rewbridge, Dodge & Ayers	—	—
Phycomycetes			*Rhytisma sp.*	—	—
(Mucorales are not included)			*Cucurbitaria sp.*	—	—
Entomophthora sphaerosperma Fres.	—	—	*Scleroderris sp.*	—	—
Empusa sp.	—	—	*Dermatea livida* B. & Br.	—	—
Dictyuchus sp.	—	—	*Biatorella resinae* (Fries) Mudd.	—	—
Pythium aphanidermatum (Eds.) Fitz.			*Glomerella rufomaculans* (Berk.) S. & S.	—	—
Phytophthora sp.	—	—	*Eutypella sp.*	—	—
(Mucorales)			Basidiomycetes		
Absidia caerulea Bainier	xxxx	xxxx	*Sorosporium relianum* (Kühn) McAlpine	—	—
Absidia glauca Hagem.—plus	xxx	xxx	*Entyloma Menispermi* Farl. & Trel.	—	—
Absidia glauca Hagem.	xxx	xxx	*Tilletia laevis* Kuehn	—	—
Rhizopus nigricans Ehren.—plus	xxxx	xxxx	*Clitocybe illudens* (Schw.) Sacc.	—	—
Rhizopus nigricans Ehren.—minus	xxxx	xxxx	*Pleurotus ulmarius* (Bull.) Fries	—	—
Sporodinia grandis Link.—minus	xxxx	xxxx	*Hymenochaete sp.*	—	—
Circinella umbellata van Tiegh. & Le Monnier	xxxx	xxxx	*Irpex tulipifera* Fries	—	—
Mucor sp.	xxxx	xxxx	*Fistulina hepatica* Fries	—	—
Mucor genevensis Ledn. (Mutant)	xxx	xxxx	*Fomes applanatus* (P.) Wallr.	—	—
Zygorhynchus sp.	xxxx	xxxx	*Trametes Pini* (Thore) Fries	—	—
Lichtheimia sp.	xxxx	xxxx	*Polyporus cinnabarinus* (Jacq.) ex Fr.	—	—
Helicostylum elegans Corda	xxx	xxxx	*Polyporus hirsutus* (Wulf.) Fries	—	—
Thamnidium elegans Link.	xxx	xx	*Daedalea quercina* (L.) P.	—	—

Chaetostylum Fresenii van Tiegh. & Le Monnier	xxx	xx	Fungi Imperfecti		
Pilobolus sp.	xx	xxxx	*Sepedonium chrysosperma* (Bull.) Fries	—	—
Blakeslea trispora Thax.—plus	xxxx	xxxx	*Oedocephalum sp.*	—	—
Blakeslea trispora Thax.—minus	xxxx	xxxx	*Arthrobotrys superba* Corda	—	—
Lymania sp.	xxxx	xxxx	*Coemansia reversa* van Tiegh.	—	—
Chaetocladium Brefeldii van Tiegh. & Le Monn.	xx	x	*Verticillium agaricinum* (Link) Corda	—	—
Mortierella I (dead wood)	xxxx	xxxx	*Penicillium glaucum* Link	—	—
Mortierella II (Brazil nut)	—	—	*Gliocladium sp.*	—	—
Mortierella III (Seattle, Wash.)	x	x	*Aspergillus candidus* (P.) Link	—	—
Mortierella IV (yellow)	—	—	*Clonostachys Gneti* Oudem.	—	—
Mortierella V	—	—	*Alternaria sp.*	—	—
Mortierella echinulata Harz	—	—	*Macrosporium sp.*	—	—
Choanephora infundibulifera (Currey) Sacc.	?[c]	xx	*Botrytis sp.*	—	—
Choanephora cucurbitarum (Berk. & Rav.) Thaxter—plus	?	x	*Cephaliophora tropica* Thax.	—	—
Choanephora cucurbitarum (Berk. & Rav.) Thaxter—minus	?	xxx	*Trichurus sp.*	—	—
Cunninghamella echinulata Thax.—plus	?	xx	*Dematium pullulans* Bary	—	—
Cunninghamella echinulata Thax.—minus	?	xx	*Cladosporium herbarum* (Pers.) Link	—	—
Piptocephalis sp.	—	—	*Isaria sp.*	—	—
Syncephalastrum I (Porto Rico)	xxxx	xxxx	*Coremium sp.*	—	—
Syncephalastrum II (Panama-sloth dung)	xxxx	xxxx	*Fusarium sp.*	—	—
			Sphaeropsis Ellisii Sacc.	—	—
			Pestalotia funerea Desm.	—	—

[a] Acknowledgment is made at this time to those who kindly gave the writer cultures of various fungi for use in this investigation.

[b] Explanation of symbols: No growth = —; Trace = x; Meagre = xx; Fair = xxx; Good = xxxx.

[c] In the case of these particular hosts, it was difficult to distinguish the vegetative hyphae of the parasite from those of the host because of their macroscopic similarity.

A. Sporangial forms:

Mucoraceae—*Absidia glauca, A. caerulea, Rhizopus nigricans, Sporodinia grandis, Circinella umbellata, Mucor* sp., *Mucor* sp. (Norway), *Mucor genevensis* (Mutant),[3] *Lichtheimia* sp.

Thamnidiaceae—*Helicostylum elegans, Thamnidium elegans, Chaetostylum Fresenii*

Pilobolaceae—*Pilobolus* sp.

Mortierellaceae—*Mortierella* I, *Mortierella* III

B. Conidial forms:

Chaetocladiaceae—*Chaetocladium Brefeldii*

Choanephoraceae—*Choanephora infundibuliformе, C. cucurbitarum, Blakeslea trispora, Cunninghamella echinulata, Lymania* sp.

Cephalidaceae—*Syncephalastrum* I (Porto Rico), *Syncephalastrum* II (Panama).

Although *D. cornuta* was not equally parasitic on all species of the Mucorales, it was observed to be equally parasitic on the plus and minus strains[3] of *Rhizopus nigricans, Absidia*

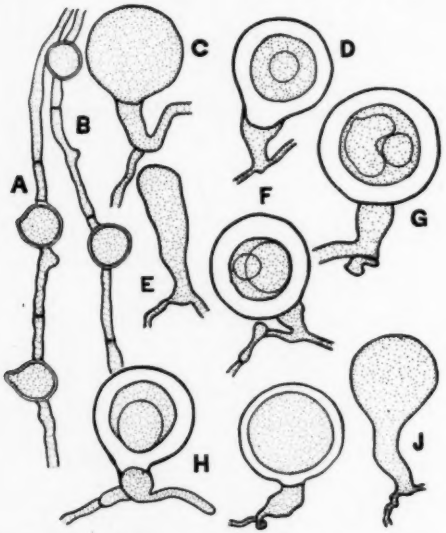

FIG. 2. A, B, chlamydospores formed by *D. cornuta* on "Difco" bacto-wort agar. 1000 ×; C, G, zygospore-like bodies formed by *D. cornuta* growing on *Mucor* sp. 1700 ×; D, F, H, mature zygospore-like bodies formed by *D. cornuta* growing alone on egg medium. 1700 ×; E, I, various stages in the formation of zygospore-like bodies when *D. cornuta* grew as a parasite on *Mucor* sp. on potato-deer dung agar. 1700 ×; J, immature zygospore-like body formed by *D. cornuta* growing alone on egg medium. 1700 ×.

[3] Obtained from Dr. A. F. Blakeslee.

glauca, *Cunninghamella echinulata*, and *Blakeslea trispora*, but more parasitic on the minus than on the plus strain of *Choanephora cucurbitarum*.

It is interesting to note that *D. cornuta* although parasitic on members of the Mucorales is very limited in its parasitism on members of the genus *Mortierella*. For example, it was found to be actively parasitic on *Mortierella* I, weakly parasitic on *Mortierella* III, but not parasitic at all on *Mortierella* II, *Mortierella* IV, *Mortierella* V, and *M. echinulata* or on various collections of the latter fungus from different sources.

In addition, *D. cornuta* was found incapable of using *Piptocephalis* sp. as a host. Similarly *Dispira* was found not to be parasitic on *Coemansia reversa*, which by many has been considered as a member of the Mucorales. The possible bearing of these results on the taxonomic position of *C. reversa* will be discussed later.

METHOD OF INFECTION OF HOSTS

Because of the simple structure of the host and the equally simple form of the parasite, an opportunity presented itself to study such points as the method of penetration, the origin of the infecting organs, the type of the parasitic organs, and the part of the host attacked. To study these points effectively, *Sporodinia grandis* was used as a host because its mycelium could readily be distinguished from that of *D. cornuta* by the greater diameter of its hyphae, its brown color, and by its lack of the disc-like septa which occur in *Dispira*. In this study, hyphae and spores from pure cultures of both host and parasite were added to hanging drops of proteose-peptone solution (1.5%). These cultures were stored in the laboratory under conditions suitable for growth of both host and parasite. Subsequent examination of these cultures showed that the spores of *D. cornuta* germinated within 24 hours, and in some cases germinated even before they were completely separated from each other in the spore chains. The first indication of germination was the increase in the size of the spores which swelled laterally until they became globular or almost so in shape (FIG. 3, *b*, *c*, *d*). After increasing in volume several times, they then produced one or

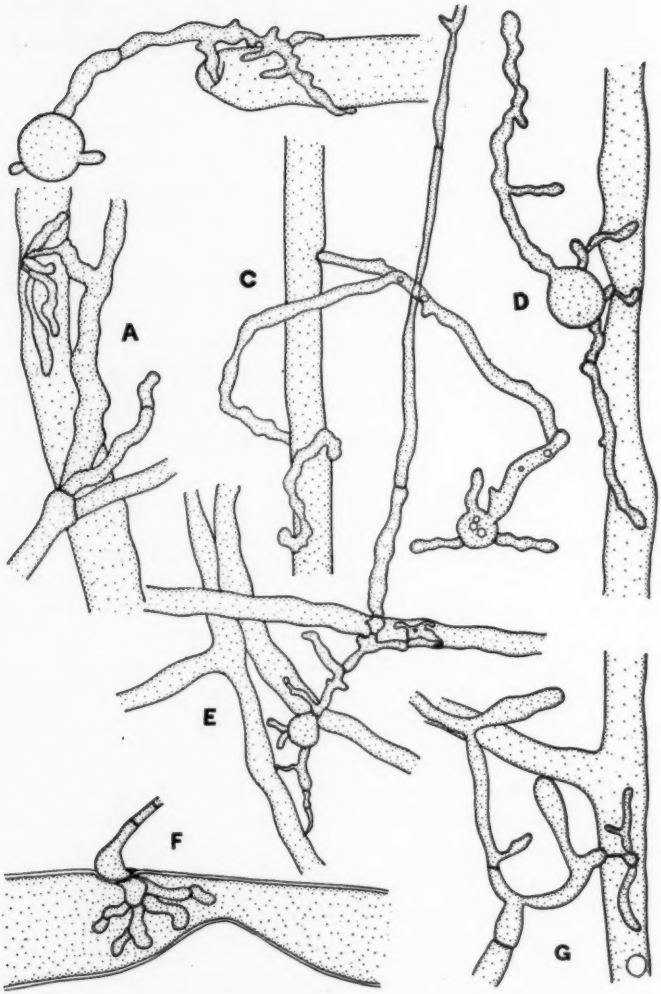

FIG. 3. A, G, haustoria formed by *D. cornuta* within the hyphae of *Sporodinia grandis*. 1700 ×; B, C, D, germ tubes from spores of *D. cornuta* beginning to penetrate hyphae of *S. grandis*. Note the germ tube pressing in the wall of the hypha of the host in the case of B and D. B, D 1700 ×, C 1000 ×; E, branches of germ tube of *D. cornuta* penetrating a hypha of *S. grandis*. In one instance, a haustorium has already been formed. 1000 ×; F, haustorium of *D. cornuta* in an old hypha of *S. grandis*. 1200 ×.

more usually terminal germ-tubes, or in some cases lateral as well (FIG. 3, *b*, *c*, *d*, *e*). These germ tubes lengthened, became septate and finally branched. Eventually some of the tubes or their branches reached the vegetative hyphae of the host, usually forming at the point of contact, knob-like appressoria (FIG. 3, *a*, *d*, *f*, *g*), from which a slender constricted tube entered the interior of the host. In a similar manner the fungus spread, developing its extensive mycelium, attaching itself to the hyphae of the hosts, and forming more haustoria within the host cells.

Once penetration within the wall of the host has been accomplished, the penetrating tubes branch to form finger-like haustoria. Seemingly the fungus gains entrance into the host by a combination of chemical and mechanical action, since in some instances the walls of the host have been observed to be indented (FIG. 3, *b*, *c*, *f*), at the points of entrance, while in others the wall is not indented (FIG. 3, *a*, *d*, *e*, *g*) and the hyphae of the parasite are constricted considerably when passing through the host walls to the interior. These observations on the host and parasite growing in hanging drops were corroborated also in a study of the two organisms growing together on various solid media. So far, haustoria have been observed only in the vegetative hyphae and never in the sporangiophores, zygospores, or aërial hyphae of the different hosts. Also in the hanging-drop cultures, it was found that the parasite was unable to penetrate the walls of the host, *Sporodinia grandis*, when they became thickened and brownish in color, and fully mature.

The haustoria formed by *D. cornuta* in the cells of *Sporodinia grandis* resembled somewhat those of this same parasite in *Mucor* sp. as illustrated by van Tieghem, but the mycelium of the parasite was observed not to be so sharply differentiated into the creeping position and the upright fertile hyphae shown by him. The parasitic organs resembled those of various fungous parasites of the higher plants, for example, the Erysiphaceae, Ustilaginaceae, and Uredinales rather than the complex "cup-cells" (Schröpfzelle) reported for the Mucoraceous parasites *Chaetocladium* and *Parasitella* by Burgeff (**8**) or the haustoria of the various species of *Piptocephalis* or *Syncephalis* on different hosts as illustrated by van Tieghem (**21**), Brefeld (**5**), and others.

THE INFLUENCE OF MEDIA ON THE PARASITISM OF DISPIRA CORNUTA

In studying the parasitism of *D. cornuta* on certain fungi, it was observed that *D. cornuta* either failed to parasitize these hosts or grew very meagerly on them when they were grown on agar media containing chiefly sugars as prune, oatmeal, cornmeal, and malt media. In contrast, when these same hosts were cultured on media such as nutrient bean pod and peptone agars consisting mostly of proteins or their derivatives, *D. cornuta* either completely covered the colonies of the host with its growth, or formed on them a large, conspicuous tuft of vegetative hyphae and sporophores. These results suggested that the presence of certain carbohydrates or proteins in the medium had a marked influence on the susceptibility or resistance of these hosts to *D. cornuta*. Therefore, tests were made to determine whether proteins and sugars actually affected the susceptibility or resistance of certain hosts to this mucoraceous parasite. For hosts in these experiments, *Mucor* sp. and *Mortierella* I were used because they had been found to be susceptible to *D. cornuta* and because their scanty growth did not mask that of the parasite. These hosts were cultivated on two types of agar media one of which contained only proteose-peptone and the other only dextrose as nutrients, varying quantities of these substances being used to determine if the concentration would affect susceptibility and resistance. In these tests *Mucor* sp. and *Mortierella* I were first transferred to the media and then approximately equal masses of inoculum consisting of the mycelium, sporophores and spores of *D. cornuta* were added. The results of these tests, together with the composition of the different media used, are embodied in Table III.

It is obvious from Table III that the kind of nutrient rather than the quantity of nutrient in each medium affected the susceptibility of *Mortierella* I and *Mucor* sp. to *D. cornuta*. For example with *Mortierella* as the host, *D. cornuta* completely covered the compact, irregular, colorless, bacterioid colony of *Mortierella* I (FIG. 4, *d*), with an almost solid mass of sporophores when this host was cultivated on proteose-peptone medium of different concentrations. In contrast, when this same host was

cultured on media containing only different quantities of dextrose as a nutrient, *D. cornuta* did not develop at all although the *Mortierella* formed limited mycelioid colonies about 35 mm. in diameter composed of delicate colorless hyphae only faintly visible to the unaided eye (FIG. 4, *c*).

TABLE III

INFLUENCE OF MEDIA ON THE PARASITISM OF DISPIRA CORNUTA

Medium used	Growth of *D. cornuta* on *Mortierella* I	Growth of *D. cornuta* on *Mucor* sp.
Proteose—Peptone Agar [1] (4, 8, 12 or 16 gms. of nutrient per L.)	*D. cornuta* covered completely the bacterioid colony of *Mortierella* I with a dense growth of mycelium and sporophores (Fig. 4, *d*).	*D. cornuta* formed a patch of sporophores about 3–4 mm. in diam. on the dense, brown colony of *Mucor* sp. (Fig. 4, *a*).
Dextrose Agar [1] (4, 8, 12 or 16 gms. of nutrient per L.)	No growth of *D. cornuta* *Mortierella* I formed a colony about 35 mm. in diam., composed of delicate, hyaline hyphae, scarcely visible to the naked eye (Fig. 4, *c*).	No growth of *D. cornuta* *Mucor* sp. covered entire surface of medium with a grayish, wooly mycelium (Fig. 4, *b*).

[1] 2 percent agar used.

Similar results were secured when *Mucor* sp. and *D. cornuta* were cultivated on dextrose and proteose-peptone media. Here again the nutrient rather than the quantity used in the media affected the susceptibility of *Mucor* sp. to *D. cornuta*. When this host was grown on media containing proteose-peptone in different quantities, *D. cornuta* made on it rather limited growth in patches about 4 × 3 mm. in diameter (FIG. 4, *a*) while the *Mucor* sp. itself covered the entire surface of the medium (40 mm. in diam.) in all cases with a compact mycelial mat which was gray at first but became light brown with sporangial production. In contrast, *D. cornuta* failed to develop at all when *Mucor* sp. was growing on dextrose media while the host spread over the entire surface of this type of substratum, forming a colony about 40 mm. in diameter, with a pale, smoke-gray color (FIG. 4, *b*), again demonstrating that the kind of nutrient rather than the quantity affected the susceptibility of a host to *D. cornuta*.

To determine if the *D. cornuta* affected the growth of either *Mucor* sp. or *Mortierella* sp. on these media, a separate series of cultures of these hosts were started from the same source simul-

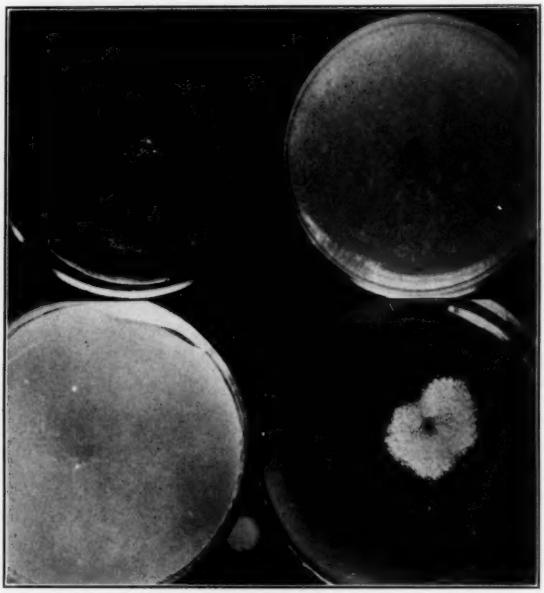

FIG. 4. Upper left, *D. cornuta* growing parasitically on *Mucor* sp., proteose-peptone agar used; upper right, *Mucor* sp. growing on dextrose agar, note the absence of *D. cornuta;* lower left, *Mortierella* I on dextrose agar, note the absence of *D. cornuta;* lower right, *Mortierella* I on proteose-peptone agar. Note that *D. cornuta* has completely covered the colony of this host.

taneously with the inoculated cultures. In these it was observed *Mucor* sp. and *Mortierella* sp. produced colonies of the same type and approximately the same size as in the cultures inoculated with *Dispira*, thus demonstrating that although *D. cornuta* is parasitic on these hosts, it does not impede their growth.

After the foregoing experiments had been repeated several times and the same results had been secured, it seemed conclusive that the composition of the medium affected the susceptibility and resistance of *Mortierella* sp. and *Mucor* sp. to *Dispira cornuta*.

However, further tests were made to determine if these conclusions were true for other organisms. In these later experiments *Mucor genevensis* (Mutant) and *Mucor* sp. (Norway) were used as hosts and cultivated on 1 per cent dextrose and 1 per cent proteose-peptone agar media (2 per cent agar). In the proteose-peptone medium, *D. cornuta* almost completely covered the bacterioid colony (25 mm. in diam.) of *M. genevensis* while on the semi-bacterioid colony (50–52 mm. in diam.) of *Mucor* sp. (Norway), it produced a limited mass of fertile hyphae which spread over an area approximately 12–15 × 7–8 mm.

In contrast, *D. cornuta* failed to produce any fertile hyphae on *Mucor* sp. or *M. genevensis* (Mutant) when these hosts were cultured on the 1 per cent dextrose medium. It is interesting to note that the growth of these two species of *Mucor* on the above media was markedly different, for on the proteose-peptone medium, *M. genevensis* (Mutant) formed a dense, bacterioid colony, cream colored, 25 mm. in diam., while on the dextrose medium it produced an open, colorless, plumose type of growth which was about 15 mm. in diam., and *Mucor* sp. (Norway) on the dextrose medium produced an open smoke-gray growth which covered the entire surface of the substratum (66 mm. in diam.) while on the proteose-peptone, it formed a more compact, bacterioid colony (50–52 mm. in diam.).

CONSTANCY OF IMMUNITY OF MORTIERELLA ECHINULATA TO DISPIRA CORNUTA

Although most of the Mucorales species tested in this investigation were found to be hosts of *D. cornuta*, *Mortierella echinulata* and several unidentified species of *Mortierella*, and *Piptocephalis* sp. were found to be immune to this parasite. An attempt to alter the immunity of *M. echinulata* was made by growing it on a proteose-peptone medium which had been demonstrated in a previous experiment to affect the susceptibility of *Mucor* sp. and *Mortierella* sp. to *D. cornuta*. This medium consisted of 8 grams of peptone, twenty grams of agar, and one liter of distilled water. To this medium transfers from pure cultures of *Mortierella echinulata* were made after which the host was inoculated with spores and mycelium from pure cultures of *D. cornuta*.

These cultures were stored in a place favorable for the growth of both organisms and kept under observation for several weeks. During this period *M. echinulata* grew so vigorously it covered the entire surface of the medium in the Petri dishes yet *D. cornuta* never developed on this organism under these conditions. Since this experiment was repeated several times, and *D. cornuta* was never observed growing in these cultures, it seems justifiable to conclude that the immunity of *Mortierella echinulata* sp. was not affected by cultivating the organism on a type of medium which had been previously demonstrated to favor the susceptibility of other organisms to the parasite under discussion.

Since all attempts to break down the resistance of the host failed, an effort was made to increase the pathogenicity of *D. cornuta* by growing this parasite first on a susceptible species, *Mortierella* I, then on a somewhat resistant form, *Mortierella* III, and finally transferring it to the immune *M. echinulata*. Following this procedure, numerous attempts were made to infect *M. echinulata* with *D. cornuta* but they were unsuccessful. The pathogenicity of the parasite did not increase as a result of development on such "bridging" hosts.

DISCUSSION

This investigation on the parasitism of *Dispira cornuta* van Tiegh. brings out some points of interest in connection with its identity, its morphology, and its parasitism.

The identity of *D. cornuta* has been confused considerably since it was first described. Although the fungus had been reported but four times in the past, it had been listed three times under the following different names, *D. cornuta*, *D. americana*, and *D. circinata*. As previously pointed out, this confusion in the identification of the fungus had resulted mainly from erroneous descriptions and inaccurate illustrations of the fungus, and from the failure to realize that it is very plastic in culture under different external conditions. For example, if van Tieghem had illustrated the *D. cornuta* correctly, Thaxter probably would not have considered the American fungus to be a distinct species. Still it must be admitted that if Thaxter had examined authentic material of van Tieghem's fungus, he undoubtedly would have

seen that he and van Tieghem had the same organism. Yet for a fungus with such distinct morphological characteristics, accurate drawings should be sufficient for its identification. Bainier's explanation that van Tieghem studied the fungus in earlier stages of development than those seen by Thaxter is unreasonable because an examination of Thaxter's plate for *D. americana* shows that he examined the fungus in all stages of development, from the formation of the primary sterigmata to the maturation of the spores. Similarly Elliott, in the case of *D. circinata*, failed to observe that this fungus is very plastic in culture and produces a variable number of branches. At the time she described *D. circinata*, Elliott apparently was unaware of Bainier's emended description of *D. cornuta* because she compared *D. circinata* with *D. cornuta* as described and illustrated by van Tieghem and pointed out the differences between these supposedly different species. Thus after considering the characters upon which the different species of *Dispira* were delimited and after studying the fungus in culture it is certain that there is only one species of *Dispira* known, and that is *D. cornuta*.

The exact position of this fungus in classification has never been definitely decided and it has been included among the Phycomycetes, Ascomycetes, and Fungi Imperfecti. To date, this fungus has never been connected with a sexual stage, although Thaxter (**18**) reported finding zygospores which he later decided were the feeding cells of *Parasitella* on *Mucor*. However, Thaxter (**18**) believed that *D. cornuta* was a member of the Mucorales because of its general habit, the peculiarities of the sporophores, the coherence of the spore mass when ripe, and the peculiarities of the septa which are common to both *Dimargaris* and *Dispira*. Van Tieghem (**21**), on the contrary, believed this aberrant group of fungi, *Dispira*, *Dimargaris*, *Coemansia*, *Kickxella*, and *Martensella*, were members of the Ascomycetes because he claimed that he had found perithecia in his *Kickxella* cultures. However, Thaxter, who had cultivated several of these forms in pure culture for many years, never found any ascigerous stages whatever. Likewise the writer has never observed *Dispira cornuta* or *Coemansia reversa* producing a sexual stage in culture

under different conditions, either when they were cultivated alone or when they were in the presence of various hosts. This of course is negative evidence and it may be that the necessary conditions for production of the sexual stage were not provided. Yet even though lacking such definite proof as zygospores, there are certain points which suggest that *Dispira* probably is a member of the Mucorales. For example, as illustrated by Thaxter (**18**), although he did not emphasize the fact in his early work on the same fungus, the asexual spores are sporangiospores instead of conidia because an outer wall can be detected around the outside of the spores, making, instead of a conidial chain, a reduced linear sporangium similar to those of *Piptocephalis*, *Syncephalis* and *Syncephalastrum*. Similarly the sporangia are borne on a head as in *Piptocephalis*, although in *Dispira* this is complicated by the presence of primary and secondary sterigmata. In addition to these points, the fertile hyphae of *Dispira* are similar in form to those of *Piptocephalis*, as in both cases they are upright, arise from a creeping mycelium which is parasitic on members of the Mucorales and are septate from the earliest stages of development. The writer has observed that septa are formed soon after germination in the germ tube of both organisms, in contrast to most members of the Mucorales, which are coenocytic or at least not septate until fully mature. Yet *D. cornuta* cannot be excluded from the Mucorales on this basis. Furthermore, the presence of *D. cornuta* in the same habitat as *Piptocephalis* and other members of the Mucorales, its early appearance in culture, on such substrata, and the fact that *D. cornuta* like species of *Piptocephalis* is parasitic only on fungi which are members of the Mucorales suggest that *D. cornuta* belongs in the same order.

Like *D. cornuta*, *Coemansia reversa* has been considered in the past as one of the Phycomycetes, Ascomycetes, and Fungi Imperfecti. Therefore, it was tested as a host for both *Dispira cornuta* and *Piptocephalis* sp. because these fungi so far have been found to be parasitic only on members of the Mucorales. As shown in Table II, *C. reversa* was not a host for either *D. cornuta* or *Piptocephalis* sp. However, these results neither prove nor disprove that *C. reversa* belongs in the Mucorales since there are

a very few members of the order, such as species of *Mortierella*, which are not hosts for *D. cornuta* or *Piptocephalis* sp. On the other hand, if *C. reversa* were a host for *D. cornuta* and *Piptocephalis* sp., this positive evidence would seem sufficient proof that *C. reversa* belongs in the order since, as previously mentioned, only members of the Mucorales have been demonstrated to be hosts for these parasites. Although the negative evidence is of course not conclusive, it seems to indicate that *C. reversa* is probably not one of the Mucorales.

The host range of *D. cornuta* is interesting since, as has been demonstrated in this paper, it includes only members of the Mucorales, an order to which the fungus itself in all probability belongs. In its host range, the writer has found members of the Mucoraceae, Thamnidiaceae, Pilobolaceae, Mortierellaceae, Choanephoraceae, Chaetocladiaceae, and Cephalidaceae even though previously only species of the genus *Mucor* had been found to be hosts. This host range is far more extensive than those reported by various authors for other parasitic species of the Mucorales, comprising, for example, more genera of Mucorales than the host ranges reported by Burgeff (**6, 7**) for *Parasitella simplex* (plus and minus strains) and *Chaetocladium* sp. Burgeff found the host ranges of these two fungi included only *Rhizopus*, *Zygorhynchus*, *Sporodinia*, *Thamnidium*, *Chaetostylum*, *Pilaira*, and *Choanephora*, while he observed that no reaction occurred between *Parasitella simplex* or *Chaetocladium* sp. and the following genera: *Phycomyces*, *Mucor Ramannianus*, *Pilobolus*, *Thamnidium*, *Cunninghamella*, and *Syncephalastrum*. As shown in Table II, *D. cornuta* has been found to attack all of the above-mentioned genera with the exception of *Phycomyces*, *Pilaira*, and *Mucor Ramannianus* which unfortunately could not be secured for tests as potential hosts. Similarly the host range of *D. cornuta* includes a greater number of the Mucorales than that of any species of *Piptocephalis* reported in the researches of Brefeld (**5**), van Tieghem (**21**), Matruchot (**15**), or of others as listed in different host indices, since they include no species of the Mortierellaceae, Choanephoraceae, and Cephalidaceae.

It is significant that *D. cornuta* should be limited in its parasitism to the members of the Mucorales even though it is not

parasitic on every member of this order. One would expect that a parasite on one fungus could parasitize almost any other fungus because of the simple structure of these organisms as compared to the complex structure of the higher plants. Probably the cell walls of the immune forms cannot be penetrated by the feeding hyphae of this fungus because, as shown in the case of susceptible forms, the fungus is unable to penetrate the cell walls when they are mature. However, Howard (**10**) in his studies on the feeding habits of *Physarum polycephalum* found that this organism would not feed on various species of Mucorales but it was able to utilize different members of the Ascomycetes and Basidiomycetes as a source of food, thus demonstrating a difference between the Mucorales and other fungi in their composition. It is interesting that the same phenomena of immunity, resistance, and susceptibility should be encountered in these lower forms of plant life as in the higher because perhaps too generally it is assumed from their simplicity of form that their physiological processes are primitive. Further, this restriction of certain hosts suggests a relationship between *Dispira* and *Piptocephalis* since both organisms are restricted in their parasitism to the same group of organisms and both have been considered to be obligate parasites on this group of which *Piptocephalis* has been demonstrated by its zygospores definitely to be a member.

It is of interest, furthermore, that the composition of the media had such a decided influence on the susceptibility of certain hosts to *D. cornuta*. Perhaps these hosts when cultivated on such media form products which either are unsuitable for the food requirements of the parasite or are antagonistic to its growth. However, it is possible that the change in osmotic concentration or hydrogen-ion concentration may have some effect even though Hurd (**11**) demonstrated that there was no correlation between hydrogen-ion concentration of the expressed juice of wheat plants and the resistance or susceptibility of wheat varieties to rust, and Hursh (**12**) found no correlation between the amount of solids in the sap of wheat plants and their resistance to rust although he did find differences in the sugar content affected the resistance of some varieties. In contrast to these results,

certain investigators have found that the sugar content of wheat plants or their nutrition by sugars increased the susceptibility to certain parasites. Pantanelli (**16**) for example found that the organs of the wheat plant most susceptible to rust are those richest in sugars, acids, and soluble compounds of phosphorus and nitrogen. Similarly Trelease and Trelease (**20**) demonstrated in the case of wheat and mildew (*Erysiphe*) that mineral nutrition of the wheat leaves which were grown in test tubes was not a limiting factor in the ability of the leaves to support mildew. However, they found that dextrose, levulose, sucrose and melizitose were most effective in increasing the susceptibility of the leaves to mildew while xylose, galactose, maltose, lactose, and glycerine were less effective and arabinose, rhamnose, mannose, and the non-sugars, starch, dextrin, and inulin or alcohol and glycerine were least effective. On the other hand, Thomas and Muller (**19**) observed that sugars such as dextrose (3 per cent), galactose (1 per cent) and saccharose (3 per cent) applied to plants through the roots decreased the quantity of infection; but whether these results are due to assimilation of sugars, changes in soil flora, or soil toxicity, was not determined. This brief review of the previous investigations on the relation of sugars to susceptibility and resistance shows divergent results because the fungi used in these experiments differ in their food requirements and the different host plants elaborate various foodstuffs. Although the susceptibility and resistance of higher plants have been changed by nutrition, the present work of *Dispira* seems to be the first instance in which the susceptibility and resistance of a fungus as host have been altered by such means.

It is of interest also that the age of the host hyphae was observed to be important in the relation between *Dispira* and its host for a similar situation has been found in the case of flowering plant hosts. Leach (**13**), for example, showed that *Colletotrichum Lindemuthianum* produced smaller lesions on old plants than on young plants. Similarly Anderson (**1**), and Walker and Jones (**22**) observed that the onion is susceptible to smut (*Urocystis Cepulae*) in the seedling stage, but it is resistant after it passes the cotyledonous stage. Also other investigators have reported the same phenomena in the relationship between various other

hosts and parasites but this is probably the first report of a similar phenomenon in the case of a fungus parasitizing another fungus.

The sex of the host has been claimed by some investigators to affect the relationship between host and parasite. For example, Burgeff (**6**) reported that a plus strain of *Parasitella* would parasitize only a minus strain of *Absidia* and vice versa. He suggested as a result of his findings that parasitism might have resulted from an imperfect sex reaction. However, in the case of *Dispira*, the writer observed that this fungus parasitized equally the plus and minus strains of *Rhizopus nigricans*, *Absidia glauca*, *Cunninghamella echinulata*, and *Blakeslea trispora*. Similarly, Satina and Blakeslee (**17**), using a large number of different physiological strains of plus and minus of *Parasitella* and *Absidia*, found that the formation of galls occurred not only when the host and parasite were of opposite sex but also when they were of the same sex. But they observed that this formation of galls occurred more frequently between the opposite sexes.

This study of the parasitism in *Dispira cornuta* is only a small beginning in a field of investigation that holds many opportunities and potentialities. The writer hopes to carry this work further in the future, but even now from this initial step it is clear that this group of relatively simple fungi offers valuable and promising material for the effective investigation of certain significant problems of parasitism.

SUMMARY

1. *Dispira cornuta* van Tiegh., the fungus used in this study, is one of the less known, so-called "conidial" species of the Mucorales. It was found initially by the author associated with an unidentified species of *Mucor* growing on hog dung from Waltham, Mass.

2. The fungus previously had been reported only twice from France, once from England, and once from North America, and hence had been considered a rare species. The writer has collected it six times since learning the requirements of the fungus.

3. Three species, *Dispira cornuta*, *D. americana* and *D. circinata*, have been reported but an intensive study of the writer's

fungus on its natural hosts and in pure culture for over two years has shown the range of variation in structural features to include the various characteristics emphasized as distinctive in the previous descriptions and illustrations. It seems justifiable to assume, therefore, that the three species are in reality reducible to one.

4. *D. cornuta* was found to be parasitic on members of the Mucorales only although a long list of fungi representing not only other orders of the Phycomycetes, but also Myxomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti were tested as possible hosts.

5. Within the Mucorales, the fungus was found to parasitize representatives of all genera tested although it was not equally parasitic on the different species used.

6. The fungus is equally parasitic on the male (minus) and the female (plus) strains of such heterothallic fungi as *Rhizopus nigricans*, *Cunninghamella echinulata*, *Blakeslea trispora*, and *Absidia glauca*.

7. In comparison with other parasitic members of the Mucorales, *D. cornuta* has a more extensive range of hosts than either *Parasitella* or *Chaetocladium* and one which is fully equal to that of the several species of *Piptocephalis*.

8. Neither *D. cornuta* nor *Piptocephalis* were parasitic on *Coemansia reversa*, a little-known conidial fungus which by some has been considered to be a member of the Mucorales because of its aberrant methods of growth and of conidial formation, and by others a member of the Ascomycetes.

9. In the case of the genus *Mortierella*, it was found that *D. cornuta* readily attacked *Mortierella* I (an undetermined species of *Mortierella* seemingly new); was only weakly parasitic on *Mortierella* III, and apparently utterly unable to parasitize four other species of this genus.

10. On species of *Mucor* and on *Mortierella* I known to be susceptible to it, *Dispira* was never able to develop when the hosts were grown on agar media containing dextrose, but grew luxuriantly when the hosts were grown on proteose-peptone agar, an instance of the alteration of the susceptibility and resistance of a host through its nutrition, not as yet reported among the fungi.

11. The morphological details of the relation of the parasite and the host were studied and it was found that in the presence of suitable hosts the spores of *D. cornuta* swelled, produced one or more germ tubes which elongated, became septate, and branched, ultimately reaching the hyphae of the host.

12. If the hyphae of the host were young, thin-walled, and growing actively the parasite penetrated, establishing haustoria within. These developed, only in the vegetative, creeping hyphae of the host, never forming in sporangia, sporangiophores or in the aerial hyphae.

13. When the vegetative hyphae of such hosts as *Sporodinia grandis* became mature, the hyphae of the parasite were no longer able to penetrate and establish haustoria within them.

14. The general significance of some of the aspects of the parasitism of *D. cornuta* is considered in relation to other pertinent cases of parasitism reported in the literature, and its importance is discussed.

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A SPECIES OF SORODISCUS ON HETERANTHERA [1]

CLIFFORD C. WERNHAM [2]

(WITH PLATES 17, 18 AND 2 TEXT FIGURES)

The fungus discussed in this paper was first collected in Lake Champlain in August 1929 by Doctor W. C. Muenscher of the Department of Botany of Cornell University. While engaged in a biological survey for the New York State Conservation Department he observed characteristic galls on the lower portions of the stem of *Heteranthera dubia* (Jacq.) MacM., and in these found the organism constantly present. Specimens were submitted to Doctor H. M. Fitzpatrick for identification, and, because of the flat disc-like aspect of many of the spore aggregations, the organism was referred by him tentatively to *Sorodiscus* of the Plasmodiophoraceae. An American collection of this genus was of sufficient interest to warrant a more complete study, and the investigation herein presented was begun.

Additional collections of the organism have been made by Doctor Muenscher and the writer in various lakes and streams in or bordering New York State. The localities are marked on the accompanying map (TEXT FIG. 1). Observations to date indicate that the distribution of the fungus may be coextensive with that of the host. As *Heteranthera dubia* occurs on mud bottoms in water of a depth of one or two meters it is usual for the plants to be completely submerged. During low water levels they are sometimes found exposed on mud flats. The galls caused by the presence of the fungus are usually hypertrophied adventitious roots, and these are found considerably below the surface of the water at the nodes of the stem (FIG. 2). Galls occur also on the true roots, though these were not observed until 1933 when

[1] Presented before the Mycological Society of America at its second annual meeting at Boston, Massachusetts, December 29, 1933.

[2] The writer wishes to acknowledge his indebtedness to Professor H. M. Fitzpatrick, under whose continued helpful supervision this investigation was prosecuted.

collections were made of plants growing on a submerged cinder fill at the southern end of Seneca Lake (FIG. 1).

The type species of *Sorodiscus*, *S. Callitrichis* Lagerheim & Winge, occurs in Europe on at least two species of *Callitriche*.

FIG. 1. Map of New York State with indication of localities in which *Sorodiscus Heterantherae* has been collected. 1, South Bay, N. Y.; 2, Dresden, N. Y.; 3, West Haven, Vermont; 4, Missisquoi Bay, Quebec; 5, Bonaparte Lake; 6, Perch Lake; 7, Black Lake; 8, Mud Creek; 9, Chaumont River; 10, Seneca Lake; 11, Cayuga Lake; 12, Dryden Lake.

One of these, *C. autumnalis* L., occurs commonly in New York associated with *Heteranthera dubia*. It is a significant fact that, although plants of this species have been examined at every opportunity, no galls have been found on them by us in any case.

HISTORICAL

The Plasmodiophoraceae embrace a rather small group of apparently primitive organisms, living as endoparasites in the cells of higher plants.

In most cases the symptoms are expressed in hypertrophied tissues. The close relationship of the various genera included

is adequately shown by a striking similarity of their life cycles. A brief description, at this point, of the typical life cycle will serve to prepare the reader for consideration of the material presented in this paper.

The thallus at first is an intracellular, uninucleate, amoeboid protoplast (myxamoeba) which increases in size as it absorbs nutrients from the cell. The nuclear divisions which occur during the growth period are peculiar to these organisms. The term "cruciform division" is commonly applied since at metaphase the persisting nucleolus divides at right angles to the equatorial plate and the two, if viewed from the side, give the appearance of a cross. At telophase the aspect is that of a "double anchor."

As the myxamoeba increases in size and in number of nuclei it may or may not fragment into daughter myxamoebae. When growth terminates, meiosis takes place. The nucleolus disappears during the prophase of the first division as in higher plants. Following meiosis the myxamoeba is partitioned into uninucleate portions by cleavage planes. Each portion envelops itself in a wall and assumes a more or less spherical form. These spherical bodies, after the formation of the wall, are called spores. The spores may remain attached to one another in groups of definite conformation, or they may fall apart and lie free in the host cell. After passing through a period of rest the spore germinates by one or more swarmspores or myxamoebae. Whether these reinfect the host directly or first fuse to form zygotes, is in dispute.

The type of spore aggregation has afforded bases for recognition of several genera. Cook (**3**), in his monographic study, separates these genera into two groups: those in which the myxamoeba forms spores only, and those in which it gives rise to both spores and zoösporangia. The first group embraces five genera: *Plasmodiophora*, in which the spores at maturity lie free in the host cell, *Sorosphaera*, in which they constitute a hollow sphere, *Sorodiscus*, in which they lie in two closely adherent layers forming a solid flattened ball or discus, *Spongospora*, in which the spore-ball is a more or less solid sphere transversed by fissures, and *Tetramyxa*, in which the spores adhere in tetrads. *Soro-*

sphaera and *Sorodiscus*, according to Cook, are further differentiated from *Spongospora* and *Tetramyxa* by the presence in the former genera of a common soral membrane about the spore aggregation. The second group includes only the genus *Ligniera*, and in it the spores are aggregated in indefinite fashion.

The literature on *Sorodiscus* is limited and not without controversial features. The first published paper is that of Kareltschikoff and Rosanoff (**5**). They describe and figure galls on *Callitriche autumnalis* containing flat plate-like bodies of constant thickness, consisting of very small polygonal cells arranged in two layers. Kareltschikoff reports on these as microchemical structures, while Rosanoff figures a mycelial organism for which the bodies appear as the spore form. Maire and Tison (**7**) believed the mycelium of Rosanoff to have no connection with the spores and state that the organism is probably plasmodiophoraceous. It seems indeed highly likely that Kareltschikoff and Rosanoff actually collected in 1870 at St. Petersburg on *Callitriche autumnalis* the same fungus that Lagerheim (according to Winge) found later in 1893 and 1900 on *C. vernalis* in Norway. Subsequent collections on *Callitriche autumnalis* were not reported until 1907, when Ostenfeld (Anonymous **1**) encountered the organism in two localities in Sweden. Lagerheim, on the basis of his own studies, regarded the organism as a mycetozoan, and because of the characteristic arrangement of its spores in flat discs erected the new genus *Sorodiscus* to include it, naming the species *S. Callitrichis*. Lagerheim's point of view remained unsubstantiated until Winge (**10**) presented a cytological study of the organism and gave us for the first time a technical diagnosis of the genus and species. He mentions the great similarity between *Sorodiscus* and *Sorosphaera*, but maintains that recognition of the former is justifiable on the basis of the arrangement of the spores at maturity, i.e., in spore-cakes, two layers thick. Winge does not state that a common membrane exists about the spore-cake but says, "At the full maturity of the spore aggregation the spore wall divides into two layers, of which the outer one merges into that of the neighboring spores so that it gives one the impression of the spores being deposited in a common substance."

For fourteen years following the appearance of Winge's paper no other contribution on the genus was made. Karling (**6**) gives an inadequate description of a fungus found causing cell hypertrophy in *Chara contraria* and *C. delicatula* at Oberlin, Ohio. As described by him, a sporangiosorus arises from a multinucleate naked protoplast and has the appearance of a flattened disc consisting of a number of definitely operculate cells. Unfortunately his material was lost before a cytological study was completed and the organism remained unnamed until Cook (**3**) saw fit to include it as a new species of *Sorodiscus*, *S. Karlingii*. Meanwhile, on the basis of Winge's work, Fitzpatrick (**4**) had incorporated *Sorodiscus Callitrichis* in the Plasmodiophoraceae of the Chytridiales as a monotypic genus.

Cook (**2**), from specimens sent him from South Africa by Ethel M. Doidge, described in 1931 *Sorodiscus radicicolus* as causing tumors on roots of an orchard weed, *Gynandropsis pentaphylla*. The smaller size of the spore-balls and individual spores, combined with the wide difference in host relationship, is considered by Cook as providing sufficient basis for separating this fungus from the European species. He states that the spore-ball "is enclosed in a delicate membrane surrounding the wall of the spores." There is no inference that this delicate membrane is the result of the fusing of the outer walls as mentioned by Winge.

Palm and Burke (**9**) present striking data on a plasmodiophoraceous fungus infecting *Veronica americana*. So variable are the spore aggregations of this species that the authors hesitate to place it in any one of the genera *Sorosphaera*, *Spongospora*, *Ligniera*, or *Sorodiscus*. Because of this situation the authors lament the unsatisfactory taxonomic condition in the Plasmodiophoraceae and suggest that several of the genera be merged.

Basing his conceptions on *Sorodiscus Callitrichis* and *S. radicicolus*, Cook (**3**) rigidly delimits the genus *Sorodiscus*. He stresses as of generic significance the arrangement of spores in a flat spore-cake of two layers, the shape of the spores, and the common membrane enclosing the spore-cake. His readiness to incorporate the organism described by Karling indicates, however, that spore arrangement is actually in his mind the fundamental

character used in delimiting genera. His point of view is thus wholly at variance with that of Palm and Burke.

In his treatment of the Plasmodiophorales Cook (**3**) apparently overlooked a paper published in 1930 by Ostenfeld and Petersen (**8**). In it these authors erected the genus *Membranosorus* and the species *M. Heterantherae* on a species found in 1924 at Lake of the Woods, Ontario, Canada. This fungus causes hypertrophy of the adventitious roots at the nodes of the stem of *Heteranthera dubia*. These roots, when young, are whitish, but when the contained spores of the fungus ripen they grow dark in color. The spores of the fungus arise from a multinucleate myxamoeba within the host cell and as seen in section seem to adhere in a single layer lining the inner surface of its wall. It is this feature of the fungus which led the authors to coin the word *Membranosorus*. So constant was this character in the scanty material which they examined that they felt justified in creating a new genus. The fact that the host is identical with the one under consideration in this investigation, and that the fungus invades similar tissues raises the question of the identity of their organism with our own.

LIFE CYCLE AND MORPHOLOGY

The galls on the adventitious roots seem to proliferate from the point of origin of the root at the node of the host. They are dark olive brown to black in color (FIG. 2) and at their base vary considerably in size, measuring 0.5–3.0 cm. in thickness. Protruding from the body of the gall are a number of finger-like projections, 0.5–1.5 cm. in length. The galls on the true roots are somewhat lighter in color and generally smaller. At maturity they have the same contour as those on the adventitious roots. The size of the gall appears to bear no relation whatever to the stage of maturity of the fungus. Though in a few of the galls only myxamoebae are found and in others only mature sori, most of them contain both stages of the organism.

Whether the zoöspores, which cause infection, are haploid or diploid has not been determined. Sections cut 7 μ in thickness from the tips of the galls and stained either with iron-alum haematoxylin or with aceto-carmine and fast green give satis-

factory material for the study of the myxamoebae.[3] In our material these usually range from 8 μ to 28 μ along the greater axis. Longer myxamoebae reaching 70 μ in length sometimes partially line the wall of the host cell and form a band about its protoplast (FIG. 4). The aspect in section is then that which would be given if the host cell wall were completely lined as claimed for *Membranosorus*. Though the host cell often contains only a single myxamoeba, it is not unusual for several to be present.

The nuclei of the myxamoeba are rather evenly spaced throughout the cytoplasm, the number varying with the size of the protoplast. The myxamoeba, during the vegetative period, is characterized by large nuclei (FIG. 5) slightly more than 3 μ in diameter, which exhibit a cruciform appearance at metaphase (FIG. 6) or the aspect of a double anchor at telophase. No centrosomes or astral rays were observed in these division figures in our preparations. The nuclei typical of myxamoebae about to undergo meiosis (FIG. 7) are smaller, being approximately 2.5 μ in diameter. The transition from the large vegetative nuclei to these smaller nuclei was not followed. Prophases, metaphases, anaphases, and telophases of the first meiotic mitosis were plentiful in our preparations and easily recognizable. During the prophase of this mitosis the nucleolus disappears completely and a normal metaphase follows. Centrosomes have not been seen, but astral rays are evident. The number of chromosome pairs was not determined with accuracy, but is probably four, five, or six. About these nuclei at metaphase a differentiation of cytoplasm suggestive of that in spore mother cells was noted (FIG. 9). The interphase nuclei (FIG. 8) are small (approximately 2 μ in diameter) and can be distinguished by their smaller size from the nuclei of the preceding growth stages. The second meiotic mitosis has been observed, but, on account of the extremely minute figures, has not been satisfactorily studied. The metaphase figures are not cruciform, nor is the evidence of subsequent spore mother cell division clear. It is realized that imperfection in staining technique may be responsible for this

[3] Miss S. M. Allen, experimenting with this material in the microtechnique course, in the Department of Botany, obtained the best preparation using Randolph's crystal violet method [Stain Technology **10**: in press. 1935].

lack of evidence. Since all the nuclei in the myxamoeba divide simultaneously they are always of the same stage of the life cycle. Nuclei representing more than one type are therefore never found together in a single myxamoeba.

Differentiation in the cytoplasm follows the last mitosis closely, and soon cleavage planes appear between the nuclei cutting the protoplast into uninucleate portions (FIG. 10). These round up and assume definite walls which gradually thicken. A sorus of globose or ovoid spores lacking a common soral envelope thus results (FIG. 11–18). The total number of spores resulting from partition of the myxamoeba varies greatly, but is believed to be always some multiple of four.

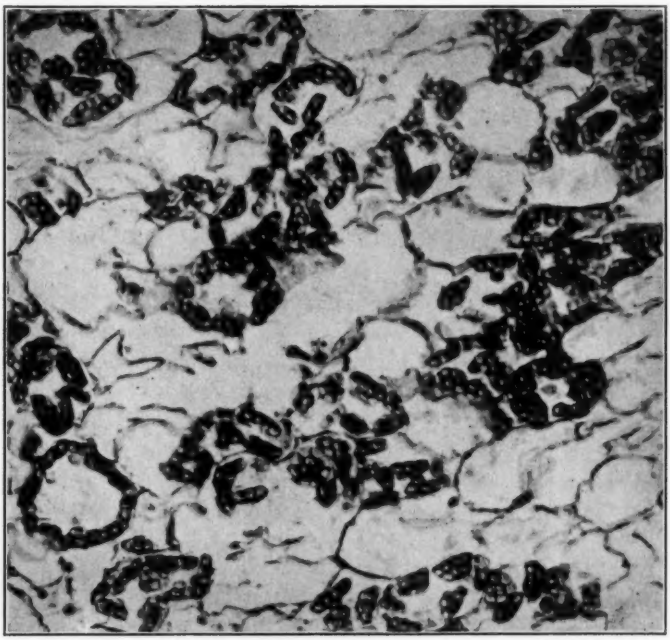

FIG. 2. Spore aggregations of *Sorodiscus Heterantherae* in the cells of the host, × 360.

The sori vary greatly with respect to the arrangement of the spores in layers (TEXT FIG. 2). Single layered sori are common

(FIG. 15, 16), but sori with two complete layers are exceptional. This latter type of arrangement is not infrequently approximated, however, by sori of the conformation illustrated in figures 14 and 18. The majority of the sori observed display an arrangement in which the greater number of spores are aligned in a single layer on which are superimposed the remaining spores of the myxamoeboid complement (FIG. 11, 12, 17). Occasionally sori were observed in which orientation into definite layers was wholly lacking (FIG. 13), the spores being aggregated merely in an irregular mass. The mature spores have thick buffy brown (Ridgeway) walls and are 3.5–4.5 μ in diameter. Occasionally slightly irregular thickening of the otherwise smooth wall has been observed, but an apical ring or collar is noticeably absent.

Time has not permitted an attempt to germinate the spores under carefully controlled conditions. During the course of the investigation another correlated problem presented itself. Large multispored sacs, which are apparently sporangia, were sometimes observed in the outer cells of the gall tissue. Whether these represent a phase in the life cycle or merely result from invasion by another fungus is not certainly known. Proof of existence of sporangial stage in the life cycle can be furnished beyond doubt only by growing the host in pure culture and by seeding the culture with spores of the organism. In the light of our present knowledge there is not sufficient reason for regarding these sacs as constituting a sporangial stage of *Sorodiscus*.

DISCUSSION

The study of the life cycle of the organism in *Heteranthera*, while not complete, has yielded sufficient data to warrant inclusion of the species in the Plasmodiophoraceae. The naked protoplast, with simultaneous nuclear divisions of the cruciform type succeeded by normal meiosis and subsequent formation of groups of uninucleate spores, presents the generally recognized cytological characteristics of the family. In addition, the organism causes marked hypertrophy of the infected tissues.

Sorodiscus was erected to include members of the family in which the spores are arranged in flat discs. The previously described species of the genus, without exception, have discs

of two spore layers in thickness. The species under consideration varies from this arrangement in that the discs are not composed consistently of two layers. Indeed so numerous are the deviates from the two-layer arrangement that these cannot be ignored. Gross dissection reveals these variants in such number that their presence in prepared slides cannot be explained as due to vagaries of microtechnique. Palm and Burke (**9**) have encountered a similar situation in *Sorosphaera Veronicae* on *Veronica americana*.

The frequent occurrence in our organism of spores in one-layered ribbons or bands (FIG. 3) has led us to believe that it is closely related to, if not identical with, *Membranosorus Heterantherae* Ostenfeld & Petersen. While not a single case of a host cell lined with a complete layer of spores, as described by them, has been observed, the condition is approached in many instances. The spores agree closely in size in the two cases. Moreover, the same tissues of identical hosts are involved in the two species with strikingly similar resultant aspects. Since these authors state that their material was extremely scanty, it is not unlikely that their species represents merely a morphological variation of our fungus.

According to Winge the spores of *Sorodiscus Callitrichis* appear to be imbedded in a matrix due to the fusion of their outer wall, while Cook states that the spore-cake of *S. radicicolus* is enclosed in a delicate soral membrane. Neither of these conditions exists in our material; nor is there present an apical ring or collar such as that figured for *S. Karlingii*. Occasionally there is some irregularity in the thickness of the wall, but in general the spores are wholly smooth. They are spherical to ovoid in shape and lack a definite axis.

Though we have not had available for comparative study material of the type species, *S. Callitrichis*, the differences evident from the description are regarded as of relative unimportance, and as insufficient to warrant exclusion of our species from the genus. As only slight broadening of the concept of the genus is necessary to include it, we propose that it be incorporated as a new species and submit the following diagnosis.

Sorodiscus Heterantherae sp. nov.

Mature thallus a multinucleate flattened naked protoplast, various in shape and size, sometimes ellipsoidal and as little as 8 μ in diameter, but more typically a disc or ribbon which not infrequently encircles the host protoplast as a definite band which may reach 70 μ in length; vegetative mitoses cruciform; meiosis occurring in the mature thallus immediately preceding cytoplasmic cleavage and spore delimitation; a soral membrane not formed; spore aggregations extremely various in size, shape, and with respect to the arrangement of the spores in layers, but total number of spores in the aggregation believed to be always some multiple of four; single-layered sori common; sori with two complete layers exceptional; intermediate types abundant; some sori failing to show differentiation into layers; others definitely ribbon-like or plate-like and often tending to line the inner surface of the host cell wall more or less completely; individual spores at maturity globose to ovoid, 3.5–4.5 μ in diameter, buffy-brown (Ridgeway), and thick-walled; spore wall smooth, not always of uniform thickness, lacking an apical ring, collar or operculum, varying in thickness from 0.6 to 1.0 μ; method of spore germination unknown; swarmspores not observed, and phase of life cycle outside of host not studied.

Parasitic in *Heteranthera dubia* (Jacq.) MacM. causing the formation of prominent dark olive brown to black galls, 0.5–3.0 cm. in diameter, on the true and adventitious roots; galls characterized by finger-like projections, 0.5–1.5 cm. in length, and containing the organism within their cells.

Occurring in various lakes and streams of the St. Lawrence basin, and perhaps elsewhere in northeastern North America.

Type material deposited at Cornell University, Harvard University, The New York Botanical Garden, Bureau of Plant Industry, and the Royal Botanic Gardens at Kew.

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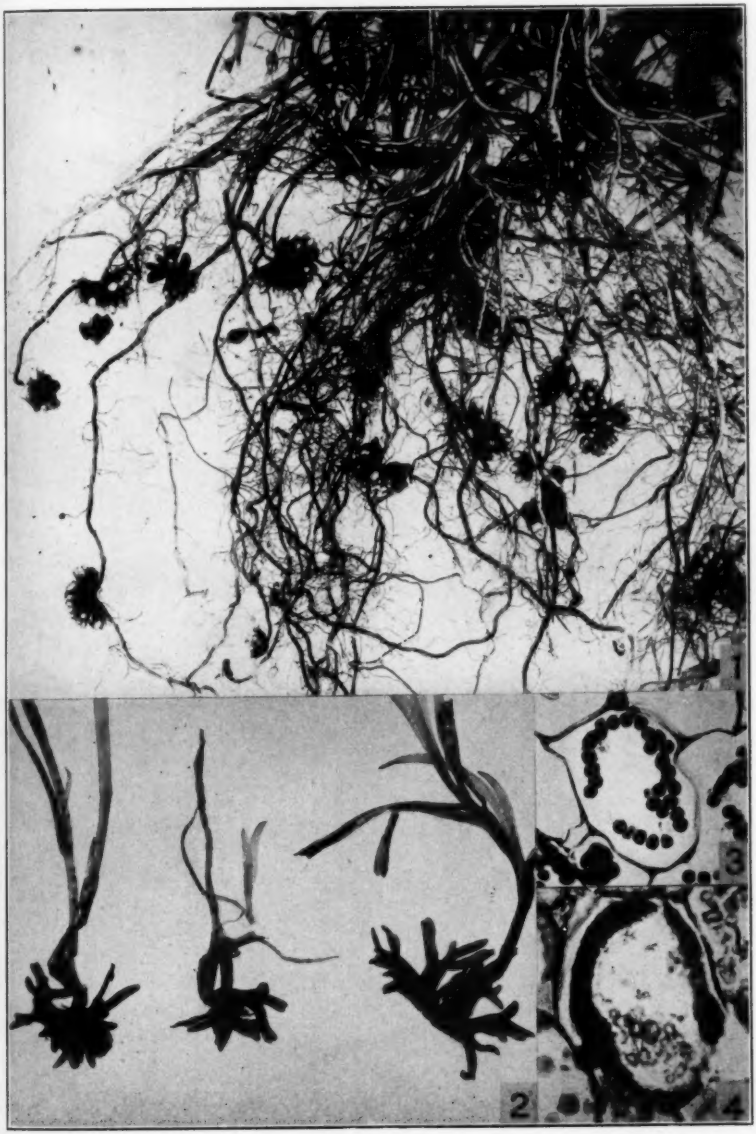

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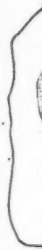

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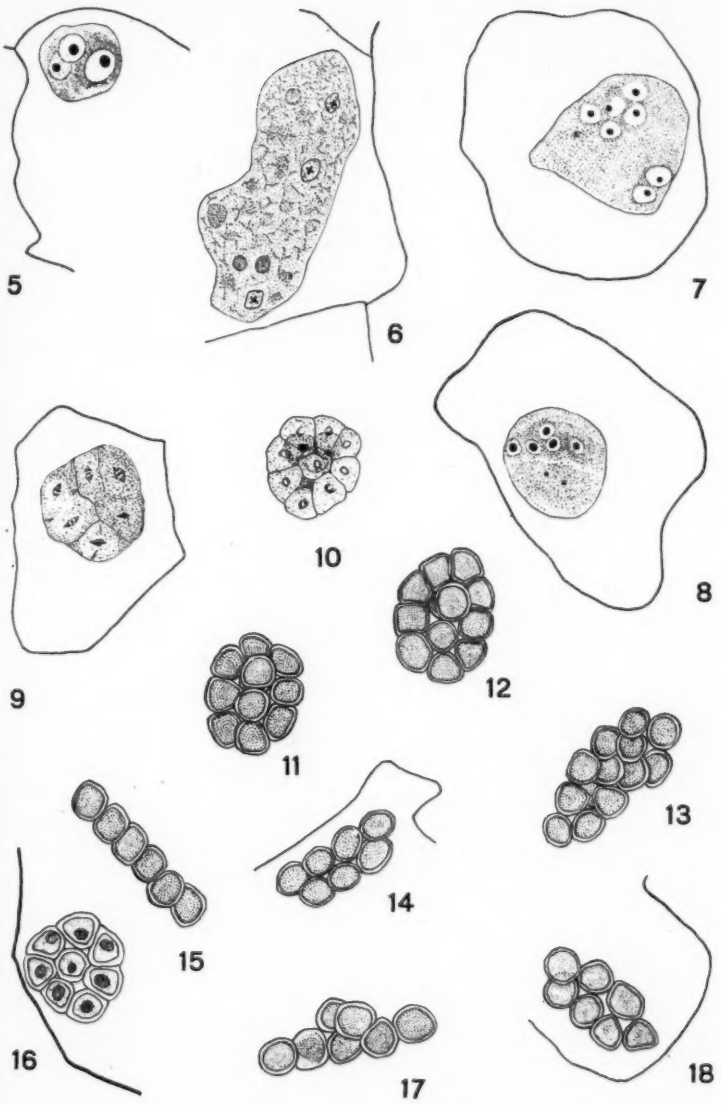

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EXPLANATION OF PLATES

PLATE 17

Photographs by W. R. Fisher. Fig. 1, galls on the true roots of *Heteranthera dubia*, nat. size; 2, galls on adventitious roots of the same host, nat. size; 3, a ribbon-like spore aggregation. × 522; 4, a long myxamoeba tending to encircle the host protoplast. × 522.

PLATE 18

The drawings were made by the author and were outlined with the aid of a camera lucida. Apochromatic lenses were used. × 1240. Fig. 5, a myxamoeba containing the large nuclei characteristic of the vegetative phase; 6, a myxamoeba showing the cruciform division figures characteristic of the growth period; 7, a myxamoeba in which the nuclei are about to undergo meiosis; 8, nuclei resulting from the first meiotic division; 9, second meiotic division accompanied by cleavage of the cytoplasm; 10, young thin-walled spores; 11, 12, disc-like spore aggregations, each composed of twelve spores; 13, spore aggregation lacking definite arrangement; 14, transverse section of a two-layered spore aggregation; 15, transverse section of a one-layered spore aggregation; 16, a single-layered eight-spored aggregation; 17, transverse section of a common type of spore aggregation in which the second layer of spores is incomplete; 18, transverse section of a two-layered sorus with open spaces between spores.

STUDIES IN THE LEPTOMITACEAE. II. CYTOLOGY OF APODACHLYA BRACHYNEMA AND SAPROMYCES REINSCHII[1,2]

ARTHUR G. KEVORKIAN

(WITH PLATES 19 AND 20)

INTRODUCTION

In the family Leptomitaceae cytological investigation had, until recently, been restricted to King's (**1903**) work on *Araiospora pulchra* Thaxter. However, in 1922 Guilliermond reported on the cytology of *Leptomitus lacteus* (Roth) Ag., and later Behrens (**1931**) on *Rhipidium europaeum* (Cornu) v. Minden. The writer has undertaken the study of representative species of the two remaining genera since a complete cytological survey of the group is desirable if the taxonomic interrelationship of the genera included in this family is to be worked out. The purpose of this paper, therefore, is to present the writer's cytological investigations of the genera *Apodachlya* and *Sapromyces*, thus making available additional data for a comparative study of the family as a whole.

MATERIALS AND METHODS

The organisms in question were collected on twigs and other vegetable substrata in ponds and ditches at various points about Boston, Massachusetts, and Kingston, Rhode Island, during the spring and fall months of the year. In order to ensure sufficient material for a cytological study of the various stages of the life cycles, the fungi were grown on corn and barberries in cooled water cultures. Changes of water were made from time to time to offset the accumulations of bacteria.

For fixation, it was found after some experimentation that Merkel's solution, as modified by Smith (**1923**), gave the most

[1] The structure and development of a new aquatic Phycomycete. Mycologia **26**: 145–152, *11 fig.* 1934, is number 1 of this series.

[2] Contribution from the Cryptogamic Laboratories of Harvard University No. 135.

satisfactory results for the hyphae and nonsexual organs. In the case of the sex organs, a weak chrom-acetic acid killing solution, consisting of 7 cc. glacial acetic acid, 7 grams chromic acid, and 1000 cc. distilled water, gave the best results, especially when fixation was carried on from 12 to 15 hours. These two solutions were therefore used most extensively, although various other dilutions of chrom-acetic, with or without osmic acid, were used, as well as Bouin's fluid.

Material killed at regular intervals throughout the day showed that the time of day at which the fungus was killed was of importance since nuclear figures were found to be more abundant in all parts of the organism during the period between 10 p.m. and 3 a.m. than at other times during the day and night.

Several staining methods were tried, including Gram's method as suggested by Couch (**1932**), Delafield's haematoxylin, according to Chamberlain (**1930**), and Heidenhain's iron-alum-haematoxylin, with or without counterstains such as eosin and light green. Each of these stains was useful for the study of certain structures, since Gram's stain gave nuclear details, whereas haematoxylin stained other cytoplasmic details as well as the nucleus. Staining, dehydration in a graded alcohol series, clearing in xylol, and embedding in paraffin were accomplished in the usual manner, except that shorter intervals (30 min.) were used in dehydration, since it was found this could be done without causing plasmolysis or any other perceptible ill effects in the material in question.

Serial sections of material embedded in paraffin were cut 5, 10, or 12 μ thick of all the developmental stages of the organism; furthermore, portions of the thalli and asexual organs were mounted "in toto," as such mounts showed some gross features especially well, and moreover were helpful in interpreting the structures found in the sections.

In the case of exceptionally heavy walled structures into which paraffin penetrates with difficulty, structures such as the mature oospores of *Sapromyces*, the best results were obtained by embedding in celloidin in the manner outlined by Jeffrey (**1928**) and Wetmore (**1932**). Although celloidin [1] has not hither-

[1] The writer is greatly indebted to Dr. H. W. Jensen and Mr. W. A. Crooks for helpful assistance in the use of the celloidin technique.

to been used to any extent for the study of aquatic fungi, the writer felt it merited a trial because of its successful use in embedding heavy walled cells of higher plants which present difficulties similar to those encountered in the mature oöspores of *Sapromyces*.

OBSERVATIONS

APODACHLYA BRACHYNEMA (HILD.) PRINGSHEIM

Of the genus *Apodachlya*, the two species *A. pyrifera* and *A. brachynema* were collected and the latter studied in detail in order to cast further light on the question of whether the "Dauersporangien" should be interpreted as gemmae, as they were by Pringsheim (**1883**), or interpreted as oögonia, as has been done by other authors.

Thallus. The numerous nuclei scattered throughout the periphery of the protoplasm consist of a dark staining, more or less central portion, or nucleolus, about which there is a hyaline nucleoplasm, surrounded by a nuclear wall. Usually there are a varying number of linin strands radiating from the nucleolus to the nuclear wall, where they become slightly thickened at the point of attachment.

The seven to twenty-five nuclei of each segment are more numerous and usually spherical at the growing tips. This is in agreement with Smith's (**1923**) findings in the mycelium of the genus *Saprolegnia*.

Sporangia. Since no evidence of nuclear division was observed in the sporangium, the writer is led to believe that the inflow of nuclei with protoplasm from the supporting segments accounts for the number contained in the young sporangium. The six to twenty-three nuclei, which are at first scattered throughout the entire sporangium, are carried to the periphery when this organ becomes vacuolate. The nuclei are usually spherical, slightly larger than those of the mycelium, and take a somewhat deeper stain.

When spore-differentiation takes place within the sporangium, each individual nucleus is surrounded by a portion of the protoplasmic mass and separated from its neighbors by a system of clefts. The resultant densely granular, somewhat angular masses finally swell and fill the entire sporangium, and, when

completely differentiated, the zoöspores are discharged through the papilla of dehiscence.

Zoöspores. At the time of discharge, the single nucleus of each somewhat elongate zoöspore is centrally located and measures about 3 μ in diameter. However, when the zoöspore becomes typically pear-shaped, the nucleus is usually near the slightly pointed apex (PLATE 19, FIG. 9*A*) but in the ensuing stage when the zoöspore rounds up and comes to rest, the nucleus again becomes central. Later, when from the encysted primary spore the secondary biciliate, kidney shaped zoöspore emerges, the nucleus within it remains in the same central position (PLATE 19, FIG. 9*B*). In both types of spores the cilia are associated with the nucleus by slender fibrillar blepharoplasts, which, as described for *Chlamydomonas* by Kater (**1929**), perhaps remain distinct but give the appearance of being fused in a single structure (PLATE 19, FIG. 9). The nuclei are usually spherical, though occasionally angular, a condition which Cotner (**1930**) observed in this species, and Mathews in *Leptolegnia* (**1932**). Upon germination the single nucleus of the zoöspore divides repeatedly, giving rise to a multinucleate germ tube.

Sexual Organs. At the ends of short lateral branches (PLATE 19, FIG. 1) are borne spherical bodies which have been interpreted as resting bodies by some authors, while others have called them oögonia subtended by an antheridium. The cytological procedure in these structures has convinced the writer that the single terminal cell is an oögonium and the cell below is a hypogynous antheridium. On sectioning and staining this hypogynous cell, the three to four dark-staining, spherical nuclei which are scattered in the protoplasm are found to be quite similar to those of the sporangium in size and structure (PLATE 19, FIG. 1). They then undergo a simultaneous mitotic division (PLATE 19, FIG. 2) and one of the resulting nuclei enlarges, while the others degenerate and the cytoplasm becomes somewhat vacuolate. Meanwhile the membrane separating the oögonium from the antheridium displays a perforation, but as in *Brevilegnia diclina* (Cooper **1929**) and *Leptolegnia caudata* (Couch **1932**) a definite fertilization tube is lacking. The functional male nucleus, together with a portion of the protoplasmic con-

tents of the hypogynous cell, now enters the terminal oögonium (PLATE 19, FIG. 4).

Since the several nuclei of the hypogynous cell all undergo a mitotic division and one of the resulting nuclei enlarges at the expense of the others, and since this is discharged into the oösphere while the rest degenerate, these points seem to indicate that Coker (**1923**) is correct in assuming that this organ is an antheridium.

Into the spherical, developing oösphere, meanwhile, ten to twenty nuclei are carried with the inflowing protoplasm. At first the nuclei, which are from 2.5 to 3.5 μ in diameter, are scattered more or less evenly throughout the cytoplasm (PLATE 19, FIG. 1); later, when the oösphere reaches its maximum size and a delimiting membrane is laid down, the nuclei undergo mitosis (PLATE 19, FIG. 2). Although all the nuclei are in process of division simultaneously with those of the antheridium, they may not all be at the same stage of division (PLATE 19, FIG. 2, 3). All but one of the resulting nuclei are then carried to the periphery, possibly by the activity of the vacuoles. Patterson (**1927**), working with *Achlya colorata*, also found that all but one of the nuclei occupied the peripheral regions of the oögonium, and he consequently designated this phase as the peripheral stage. The nucleus which remains in the center of the oögonium then enlarges, becoming two to three times its original size, while those at the periphery degenerate (PLATE 19, FIG. 4). While this is the general rule, in some cases the nuclei have been seen to divide and degenerate without migrating to the periphery, with the exception of the one which remains in the center.

Surrounding the centrally located female gamete-nucleus there now may be seen in many cases a finely granular, not too pronounced, irregular area which is apparently the coenocentrum described by various writers in certain of the Saprolegniales and Peronosporales. Meanwhile the male gamete-nucleus is liberated by the antheridium, and travels toward the egg nucleus (PLATE 19, FIG. 4), and upon coming in contact, both enlarge (PLATE 19, FIG. 5) and become rather closely appressed. At this stage several dark-staining linear condensations of cytoplasm are seen radiating outward from the periphery of the nucleus

(PLATE 19, FIG. 6, 7). These structures have been called astral rays by various workers and are found in closely related groups such as the Saprolegniaceae and the Peronosporaceae. The spherical oösphere now contains the enlarged functional male and female nuclei and the degenerating peripheral nuclei (PLATE 19, FIG. 6) which soon disappear completely.

Fusion is evidently a slow process, since the oöspore wall may become clearly defined and increase in thickness before the male and female nuclei lose their identity (PLATE 19, FIG. 7). A similar delayed fusion was also noted by Stevens (**1901, 1902**), Trow (**1901**), and Patterson (**1927**) in representatives of related groups.

That the so-called resting sporangia or "Dauersporangien" are in reality oögonia and the sub-terminal cells antheridia seems clear, since the procedure mentioned above, namely, the division of the nuclei in the young antheridium and the young oögonium, the degeneration of the supernumerary nuclei in both organs, the subsequent migration of a single male gamete-nucleus into the oösphere, and eventually its fusion with the centrally located female gamete-nucleus, seems conclusive evidence that these bodies are sexual organs.

The fate of the fusion nucleus could not be followed during the early stages of oöspore germination since the characteristic large oil globule which usually occupies the central portion of the mature oöspore made observation difficult. Although this point has not been clarified, it may be assumed, however, that, as in the Monoblepharidales (Laibach **1927**), reduction division is one of the several divisions which occur upon germination, giving rise to multinucleate germ tubes. This is accomplished after a resting period of several weeks.

SAPROMYCES REINSCHII (SCHRÖTER) FRITSCH

Although material of the two known species of the genus *Sapromyces* was collected and subjected to a comparative study, it seemed more fitting to present a cytological account of *S. Reinschii* since previously it has been the less studied of the two.

Thallus. In the basal cell, which develops from the germinating zoöspore, the nuclei are scattered throughout the periph-

eral portion and are so numerous that an accurate count can be made only with difficulty. This multinucleate condition results from the repeated divisions of the single zoöspore nucleus during the period of germination. In shape, the nuclei are slightly elongate (3.5 to 4 x 5 μ) or spherical (4 μ in diameter) and structurally similar to those already described for *Apodachlya brachynema*. In older plants they are not so numerous and are more elongate, especially those which are found in the basal cells.

Sporangia. The large spherical nuclei are carried into the sporangia by the inflowing protoplasm from the adjacent segments, a procedure similar to that described in *Apodachlya brachynema*.

In young sporangia the large spherical nuclei are scattered throughout the entire content, but after the small vacuoles have coalesced to form a large central vacuole, the nuclei assume a peripheral position. Upon further differentiation, the 12 to 30 nuclei of the sporangium are separated into individual spore initials by a number of slender clefts of vacuolar origin. Further details of the process of spore formation are similar to those described for *Apodachlya*.

Zoöspores. The centrally located, spherical to slightly angular, single nucleus of the zoöspore (PLATE 20, FIG. 2*A*), like the nuclei of the thallus, is enclosed by a finely granular, rarely vacuolate central cytoplasmic area which is in turn surrounded by the coarsely granular, rather vacuolate, peripheral cytoplasm containing irregular densely-staining granules, and the whole surrounded by a thin limiting membrane.

The two lash-like cilia, which are about four times as long as the spore body, one directed forward, and the other backward, have their origin in the lateral groove of the zoöspore and are connected to the nucleus by an arrangement of basal granules, similar to those previously described in *Apodachlya* and also for *Rhipidium* (Cotner **1930**) and *Araiospora* (Kevorkian **1934**).

Sexual Organs. The curved, oblong antheridium twines about the oögonium and attaches itself at its apex by means of a beak-like process. In this body the four to six nuclei are similar in structure to those of the sporangia and oögonia, but after a short time they undergo one mitotic division (PLATE 20, FIG. 4) and

one of the resulting nuclei, usually more deeply-staining, enlarges, while the rest gradually degenerate. In the meantime the antheridium pushes in a beak-like process at the point of attachment. This definite, rather thick-walled fertilization tube enters the oösphere and discharges its male gamete-nucleus, allowing it to migrate toward the centrally located egg nucleus (PLATE 20, FIG. 5).

In the young oögonial initial the ten to twelve spherical nuclei which are carried in with the inflowing protoplasm are scattered throughout the protoplasm (PLATE 20, FIG. 3, 6), as in *Apodachlya;* the oögonium soon becomes vacuolate and the nuclei undergo one mitotic division, somewhat similar to that which occurs in the genus *Araiospora* (King **1903**), and all but one migrate to the periphery (PLATE 20, FIG. 4, 6). In certain other instances, however, all but the selected female gamete nucleus degenerate without going to the periphery (PLATE 20, FIG. 11, 12). Behrens (**1931**), referring to *Rhipidium*, states: "Wenn das Oogon seine volle Grösse erreicht . . . haben sich die Kerne in ziemlich regelmässigen Abständen über die ganze Peripherie verteilt, mit Ausnahme eines einzigen, der in der Mitte der Oogons verbleibt." This observation seems to agree with what the writer finds in *Sapromyces*. The remaining, more or less centrally located nucleus enlarges to from 6 to 8 μ in diameter at the expense of the others which, as stated above, degenerate (PLATE 20, FIG. 5–7), a process which also takes place in certain of the Peronosporaceae (Berlese **1897**). The separated periplasm, which usually contains the peripheral nuclei, gradually changes in texture and soon gives rise to a membrane separating the more granular oöplasm from the less granular periplasm (PLATE 20, FIG. 9). At this stage of development, as in *Apodachlya*, the so-called "coenocentrum" makes its appearance in the central portion of the oögonium (PLATE 20, FIG. 8). This finely granular and less vacuolate area soon disappears and the enlarged, rather closely associated male and female nuclei can be seen more distinctly (PLATE 20, FIG. 10, 11). After some delay these fuse, forming a much enlarged, single, centrally located nucleus in the mature oöspore (PLATE 20, FIG. 12). As is the case in numerous other watermolds, the further fate of the fusion nucleus is not known, since germination has never been observed in *Sapromyces*.

LEPTOMITUS LACTEUS (ROTH) AGARDH

The cytology of *Leptomitus lacteus* was worked out in the same detail as that of *Apodachlya* and *Sapromyces*, but since sexual organs have not been reported in the genus, the observations were necessarily confined to the mycelium and non-sexual organs. The oval to elongate nuclei, which are usually located in the peripheral protoplasm of the mycelium, are from 2 to 3 μ in diameter and somewhat similar to those of *Apodachlya* and *Sapromyces*. The nuclei within the sporangia average about twenty to thirty in number, are usually spherical, and possess a dark-staining nuclear membrane enclosing a dark, centrally located nucleolus surrounded by a hyaline zone. These findings only corroborate what has already been worked out by Büsgen (**1882**) and Dangeard (**1890**) and, being limited to the mycelium and sporangia, naturally furnish no data for comparison with the cytological details of the sexual organs or fertilization in the other genera.

CONCLUSIONS

The cytological procedure in the so-called "Dauersporangien" of *Apodachlya brachynema* reveals the fact that these structures are in reality terminal oögonia and the sub-terminal cells antheridia. Although a number of authors such as Coker (**1923**), Sparrow (**1932**), Cejp (**1932**), etc., have suggested that these bodies, in structure, position, and behavior, gave indication of being sexual organs, cytological observations to support their morphological evidence of the true identity of these terminal organs has been lacking hitherto.

Unlike the other members of the family, the sexual organs of *Apodachlya brachynema* are more closely allied to such forms as *Leptolegnia* and *Brevilegnia* of the Saprolegniaceae than those of the Leptomitaceae, in that there is no definite separation of the protoplasmic content of the oögonium into an inner oöplasm and outer periplasm. Furthermore, the oöspore of *Apodachlya*, at maturity, lacks a definite exospore such as exists in *Sapromyces*, *Araiospora*, and *Rhipidium*. In *Sapromyces*, on the other hand, the cytological procedure is very similar to that of certain members of the Peronosporales, notably *Sclerospora*, *Pythium*, *Peronospora*, etc., as studied by Stevens (**1902**), Trow (**1901**), and Ber-

lese (**1897**), in that the oögonium containing the scattered nuclei becomes vacuolate and a nuclear division takes place. Later all but one of the resultant nuclei migrate to the less granular periplasm and there degenerate. The selected male nucleus discharged by the fertilization tube then fuses with the enlarged female nucleus located in the densely granular oöplasm.

These data, together with certain morphological observations (Kevorkian, unpublished) such as the Saprolegnoid habit, diplanetic zoöspores and oöspore devoid of a definite exospore, lead the writer to believe that *Apodachlya* and its ally *Leptomitus* are transitional forms between certain members of the Saprolegniaceae and Leptomitaceae, whereas *Sapromyces* and its allies are more closely related to the Peronosporales than the Saprolegniales.

In conclusion the writer wishes to acknowledge his indebtedness to Professor W. H. Weston, Jr., under whose supervision this research was done, for his constant interest, helpful assistance, and stimulating criticism; to Dr. D. H. Linder for many valuable suggestions throughout this work; and to Dr. R. M. Whelden for helpful assistance pertaining to cytological procedure.

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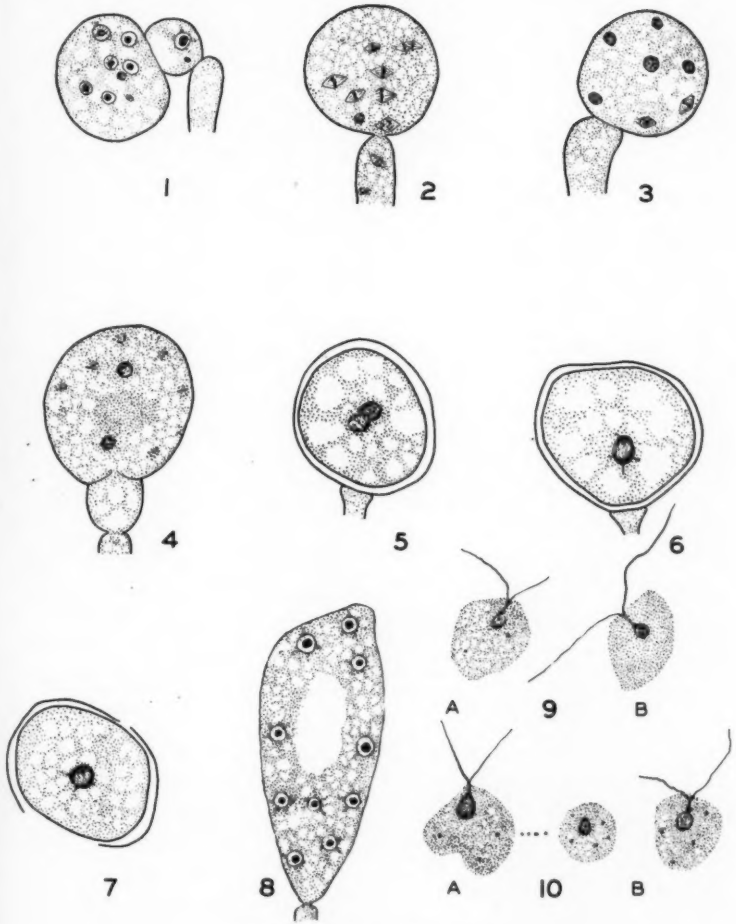
EXPLANATION OF PLATES

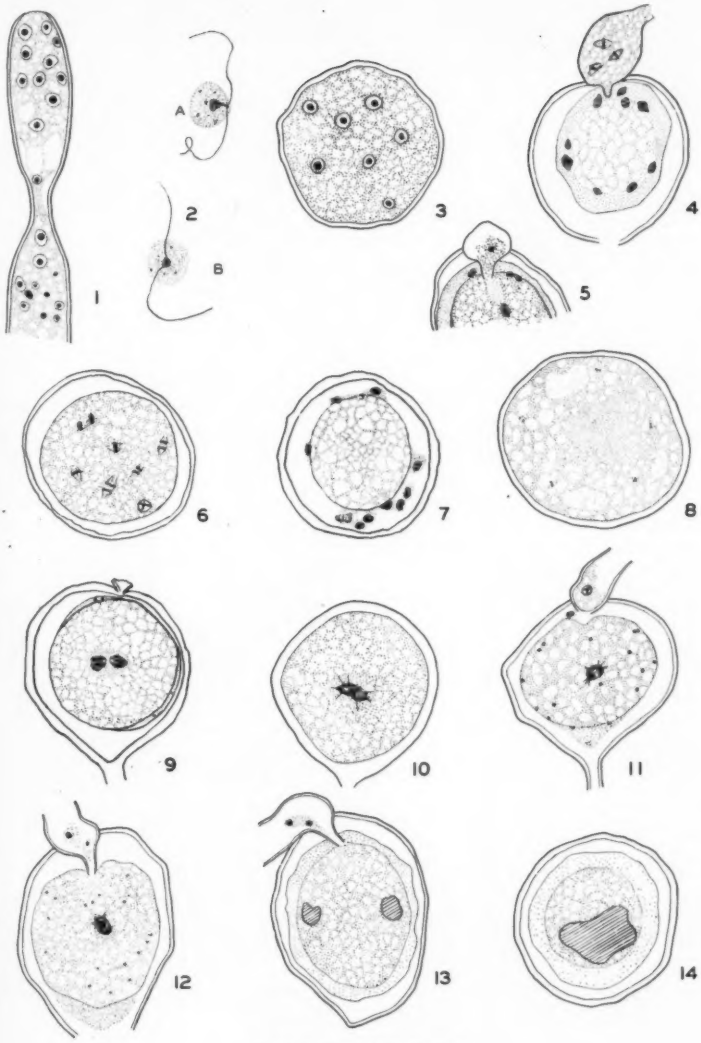
The figures were drawn from killed and fixed material with the aid of an Abbe Camera Lucida with a 10 × ocular and 1.25 mm. objective. Approximate magnification × 800 for Plate 19 and × 500 for Plate 20.

PLATE 19

Apodachlya brachynema: Fig. 1, young oögonium and antheridium showing arrangement of nuclei; 2, mitosis in the oögonium and antheridium; 3, oögonium showing peripheral arrangement of nuclei before degeneration; 4, oögonium showing male and female nuclei and degenerating nuclei at periphery. Coenocentrum is present; 5, oögonium with thickened oögonial wall, showing association of centrally located male and female nuclei; 6, oögonium showing nuclei with astral rays prior to complete fusion; 7, fusion nucleus with astral



1–9. APODACHYLA BRACHYNEMA
10. LEPTOMITUS LACTEUS



SAPROMYCES REINSCHII

rays; 8, sporangium showing arrangement of nuclei; 9, A. zoöspores of the primary type; B. zoöspores of the secondary type; 10, *Leptomitus lacteus:* A. zoöspores of the primary type; B. zoöspores of the secondary type.

PLATE 20

Sapromyces Reinschii: Fig. 1, nuclear distribution in very young sporangium; 2, A and B. zoöspores; 3, nuclear distribution in early stage of oögonium; 4, peripheral arrangement of oögonial nuclei prior to division, antheridial nuclei at metaphase; 5, migration of the chosen male nucleus; 6, oögonial nuclei dividing before migration to periphery; 7, degeneration of peripheral nuclei; 8, oögonia showing coenocentrum; 9 and 10, male and female nuclei prior to fusion; 11, male and female nuclei in close association showing astral rays, supernumerary nuclei in process of degeneration; 12, oögonium showing fusion nucleus; 13 and 14, mature oöspores showing oil globules.

CONIDIAL FORMATION, MUTATION AND HYBRIDIZATION IN PENIOPHORA ALLESCHERI[1]

MILDRED K. NOBLES

(WITH PLATES 21–23 AND 4 TEXT FIGURES)

Preliminary examination of cultures of *Peniophora Allescheri* Bres. showed that the fungus produces abundant conidia on oedocephaloid heads on both haploid and diploid mycelia. Observations of the occurrence of similar structures in the Hymenomycetes have been reported by Brefeld for *Tomentella flava* Brefeld and *T. granulata* Brefeld (**1**), for *Fomes annosus* (Fries) Cooke by Brefeld (**1**), Lyman (**6**) and others, and for *Corticium roseo-pallens* Burt and *C. effuscatum* Cooke and Ellis by Lyman (**6**), but these studies were made prior to the establishment of modern conceptions of interfertility phenomena in Basidiomycetes. Hence, in most cases, it is impossible to determine from the data whether the conidia were produced on haploid or diploid mycelia, or the value of the mycelium obtained by their germination.

Other types of conidial or oidial formation have frequently been reported among the higher Basidiomycetes, and in a few cases some analyses of the spores have been made. Accessory spores produced on the haploid mycelium repeat that generation, and in some instances spores produced on the diploid mycelium repeat that phase, as is the case in *Polyporus squamosus* Huds. and *Pleurotus pinsitus* Fries (**10**). This is not universal. Gilmore (**2**), working on *Psilocybe coprophila* Fries, showed that the diploid mycelium produced oidia, some of which gave rise to haploid, some to diploid mycelia. Martens and Vandendries (**7**), studying *Pholiota aurivella* Batsch., found that the conidia developed on specialized branches on the diploid mycelium were either diploid or haploid in value, the latter arising by the division and disjunction of a binucleate spore in such a way as to give rise to two uninucleate spores.

[1] Contribution from the Department of Botany, University of Toronto.

Since no detailed study had been made of conidial formation in a Basidiomycete having the Oedocephalum type of conidiophore, such a study was undertaken of *Peniophora Allescheri* to determine, first, stages in conidial formation and second, the value of the conidia.

		A								B		
		2	7	9	10	23	24	25	27	28	16	19	22
A	2	−	−	−	−	−	−	−	−	−	+	+	+
	7	−	−	−	−	−	−	−	−	−	+	+	+
	9	−	−	−	−	−	−	−	−	−	+	+	+
	10	−	−	−	−	−	−	−	−	−	+	+	+
	23	−	−	−	−	−	−	−	−	−	+	+	+
	24	−	−	−	−	−	−	−	−	−	+	+	+
	25	−	−	−	−	−	−	−	−	−	+	+	+
	27	−	−	−	−	−	−	−	−	−	+	+	+
	28	−	−	−	−	−	−	−	−	−	+	+	+
B	16	+	+	+	+	+	+	+	+	+	−	−	−
	19	+	+	+	+	+	+	+	+	+	−	−	−
	22	+	+	+	+	+	+	+	+	+	−	−	−

TABLE I. Results obtained by pairing in all possible combinations twelve single basidiospore cultures of *Peniophora Allescheri*. A plus (+) sign indicates the presence of clamp connections, a minus (−) sign their absence.

The fruiting body from which the cultures were originally obtained was collected on bark of *Populus* sp. by Professor H. S. Jackson, near Aurora, Ontario, on October 22, 1933 (U. of T. Herb. *No. 5605*). It was identified as *Peniophora Allescheri* Bres. by Dr. L. O. Overholts.

CULTURES FROM BASIDIOSPORES

The fruiting body yielded basidiospores which, when sown on plates of cooled malt extract agar, germinated in from two to three days. Isolated colonies were transferred to stock culture tubes containing malt extract agar and in this way twenty single basidiospore cultures were obtained.

When grown on 2 per cent malt extract agar at room temperature, the colony resulting from a single basidiospore is slow growing (FIG. 1*b*) with most of the mycelium submerged or appressed, and with scanty aerial growth. Microscopic examination shows that the hyphae have simple septa, without clamp connections. This condition has persisted through several transfers over the six months the fungus has been in culture.

While the colonies are still microscopic in size, conidial production begins. The conidia are produced on specialized conidiophores which may be either apical or lateral with respect to the hyphae. The typical conidiophore (FIG. 3, 4, 5) is a rounded or club-shaped structure 9–14 μ in diameter, at first smooth, later developing slender tapering sterigmata, up to 2 μ in length, on which the conidia are borne. The number of conidia on a head varies from eight to thirty-two, although occasional conidiophores have only four conidia. The conidiophore, which is separated from the hypha by a septum, becomes vacuolate as the spores develop, and finally empty, the contents all passing into the spores. In plate cultures the spores remain in a mass on the collapsed conidiophore; in hanging drop cultures they float away (FIG. 5).

The conidia vary in length from 12–15 μ, are hyaline, cylindrical and often somewhat curved.

Twelve haploid mycelia, each derived from the germination of a single basidiospore, were grown in pairs in all possible combinations, and the resulting mycelia examined microscopically for the presence of clamp connections. The results are incorporated in Table I,[1] in which a plus sign indicates the occurrence of clamp connections, a minus sign their absence. It will be observed that the mycelia fall into two groups, a member of

[1] In this paper the symbols A and B have been used to designate the two groups of mycelia, in place of the more conventional A and a.

group A reacting with a member of group B in such a way as to produce a mycelium bearing clamp connections. A member of one group is unable to react in this way with another member of the same group. *Peniophora Allescheri* is therefore genetically heterothallic, defining this term to mean that the thalli which react together to produce a clamp-bearing mycelium contain nuclei of different genetic constitution as regards the factors which determine for or against this reaction.

The diploid mycelium obtained by growing together two suitable mycelia, resembles the parent haplonts in rate of growth and character of colony (FIG. 2*a*) and in microscopic characters, except that the hyphae of the diploid mycelium have clamp connections at every septum. This mycelium, like the haploid, bears abundant conidiophores (FIG. 6) which are similar in appearance to those borne on the haplonts. The conidia developed on these conidiophores are not distinguishable from those produced on the haploid mycelium.

CYTOLOGICAL STUDY OF CONIDIAL FORMATION

A cytological study has been made of basidiospores, sporelings and haploid and diploid mycelium. For this purpose the material to be examined was used to inoculate a drop of 2 per cent malt extract solution on a coverslip and inverted over a van Tieghem cell. At the desired stage of development, the material was killed by adding a drop of formalin-acetic-alcohol or Bouin's fixative, and dried down on the coverslip. It was then stained in Haidenhain's haematoxylin, dehydrated and mounted in the usual way.

The basidiospore is uninucleate and germinates to produce a germ tube which becomes septate as soon as the first nuclear division occurs. Thus the sporeling and haploid mycelium are strictly uninucleate from the beginning.

The first indication that a conidiophore is forming in a terminal position is a swelling at the end of the hypha. When the conidiophore occupies a lateral position it usually attains almost its final size before the nucleus of the cell from which it arises migrates into it. In the early uninucleate stage (FIG. 9) the contents of the conidiophore are homogeneous and densely staining.

By successive divisions two, four, eight, sixteen or more nuclei are formed within the head, the nuclei following each division appearing successively smaller than those resulting from the preceding division (FIG. 10, 11, 12). The nuclei take up their

		A								B		
		1	2	5	6	7	8	9	10	11	3	4	12
A	1	−	−	−	−	−	−	−	−	−	+	+	+
	2	−	−	−	−	−	−	−	−	−	+	+	+
	5	−	−	−	−	−	−	−	−	−	+	+	+
	6	−	−	−	−	−	−	−	−	−	+	+	+
	7	−	−	−	−	−	−	−	−	−	+	+	+
	8	−	−	−	−	−	−	−	−	−	+	+	+
	9	−	−	−	−	−	−	−	−	−	+	+	+
	10	−	−	−	−	−	−	−	−	−	+	+	+
	11	−	−	−	−	−	−	−	−	−	+	+	+
B	3	+	+	+	+	+	+	+	+	+	−	−	−
	4	+	+	+	+	+	+	+	+	+	−	−	−
	12	+	+	+	+	+	+	+	+	+	−	−	−

TABLE II. Results obtained by pairing in all possible combinations twelve cultures from single conidia developed on diploid mycelium.

positions around the inner surface of the distal half of the conidiophore and the basal portion becomes vacuolate. At this stage the slender tapering sterigmata push out and a swelling, the developing conidium, appears on the end of each. While the spore is still small a nucleus migrates into it, becoming thin and elongated in order to pass through the narrow sterigma (FIG. 14). The conidium increases in size, the contents stain densely and appear homogeneous, and the nucleus increases in size (FIG. 13). Apparently all of the cytoplasm of the conidiophore is used up in

spore formation, for when all the conidia are mature, the head appears empty and finally collapses (FIG. 5).

The cells of the clamp-bearing mycelium are regularly binucleate, the nuclei undergoing conjugate divisions through the agency of the clamp connection. Conidiophore development proceeds in a manner similar to that in the haploid conidiophore. The conidiophore may occupy a terminal position, in which case it appears first as a swollen binucleate cell, or a lateral position, when it pushes out from the side wall of a cell and reaches a considerable size before the two nuclei of the cell migrate into it (FIG. 15). In either case, the conidiophore is at first binucleate (FIG. 15, 16). These two nuclei divide simultaneously but without the formation of a clamp connection (FIG. 17). Later stages show the presence of four, eight, sixteen or more nuclei in each head (FIG. 18). The formation of sterigmata and spores takes place as in the haploid conidiophores, and one nucleus migrates into each conidium. Thus the binucleate, clamp-bearing diploid mycelium produces uninucleate conidia (FIG. 19). The evidence from cytological study indicates that both the original nuclei have taken part in providing the daughter nuclei that finally migrate into the spores.

CULTURES OF CONIDIA FROM DIPLONT

As previously stated, the conidia produced on the binucleate mycelium are themselves uninucleate. This observation is substantiated by the results of culture work.

A pairing of single basidiospore cultures 1 and 12 was made by inoculating a plate of cooled malt extract agar with mycelium from each of the stock cultures. They grew together, the resulting mycelium bearing clamp connections. A transfer of hyphal tips from this clamp-bearing mycelium was made to an agar slant in a stock culture tube. Later a transfer from this diploid stock culture (1×12) was made to a plate of cooled malt extract agar. By this method, the possibility of haploid mycelium being carried over was reduced to a minimum.

Conidia are produced very abundantly on such a diploid colony. These conidia were used to inoculate hanging drop cultures of 2 per cent malt extract solution, in order to watch

germination. They germinated in from twenty-four to forty-eight hours at room temperature, to give sporelings with simple septa, indistinguishable from the sporelings derived from basidiospores. This indicated that the conidia produced on the

		A	B
		1	12
A	2	−	+
	7	−	+
	9	−	+
	10	−	+
	23	−	+
	24	−	+
	25	−	+
	27	−	+
	28	−	+
B	16	+	−
	19	+	−
	22	+	−

TABLE III. Results obtained by mating twelve single conidial cultures from a diploid mycelium with the two parent basidiospore cultures.

diploid clamp-bearing mycelium germinate to give haploid mycelia.

That this is the case was proved by further work. Conidia from this diploid colony (1 × 12) were sown on plates of cooled malt extract agar, and isolated colonies were transferred to agar slants in stock culture tubes. In this way twenty-five single conidial cultures were obtained. These cultures (with one exception to be discussed later) are identical with the cultures arising from single basidiospores, in gross appearance and absence of clamp connections.

Twelve of these single conidial cultures were grown in pairs in all possible combinations, and the resulting mycelia examined for the presence of clamp connections. The results are given in Table II where as before the plus sign indicates the presence of clamp connections, the minus sign indicates their absence. It will be observed that, as in Table I, the mycelia fall into two groups, a member of one group reacting with a member of the other group in such a way as to produce a mycelium bearing clamp connections.

Each of the twelve single conidial cultures was then paired with basidiospore culture 1 and basidiospore culture 12 and the resulting mycelia examined for the presence of clamp connections. The results are shown in Table III from which it is evident that conidial cultures 16, 19 and 22 are identical with basidiospore culture 12, and so belong to group B, while conidial cultures 2, 7, 9, 10, 23, 24, 25, 27 and 28 are identical with basidiospore culture 1 and so belong to group A.

While the cytological study indicated that both nuclei of the young conidiophore divide to provide the nuclei that migrate into the spores it seemed desirable to test this by determining whether the conidia from a single head are of the two kinds. Considerable difficulty was encountered in removing the spores from a single conidiophore, since the conidiophores were very closely crowded on the colony, and the spores were repelled by, or at least did not cling to, a fine steel needle which was used in the first attempts. Later it was found that the conidia could be successfully transferred by using a fine glass needle rubbed with silk in order to induce an electric charge. The spores were apparently attracted to this, and several conidia from one head could be removed to the surface of poured malt extract agar plates. Spores from eight single conidiophores borne on diploid clamp-bearing mycelium, have been allowed to germinate in groups, and in every case clamp-bearing mycelium has developed. This result clearly indicates that the conidia from a single conidiophore were of two kinds, and gave rise to two kinds of mycelia which were able to react in such a way as to produce diploid clamp-bearing mycelium.

As a result of cytological and cultural work, it may be con-

cluded that the diploid clamp-bearing mycelium produces conidia which are haploid in nature, that these conidia germinate to give haploid mycelia, and that these haploid mycelia react with one another and with the mycelia derived from single

		A						B					
		18	19	20	21	22	23	1	2	3	4	5	6
A	18	−	−	−	−	−	−	+	+	+	+	+	+
	19	−	−	−	−	−	−	+	+	+	+	+	+
	20	−	−	−	−	−	−	+	+	+	+	+	+
	21	−	−	−	−	−	−	+	+	+	+	+	+
	22	−	−	−	−	−	−	+	+	+	+	+	+
	23	−	−	−	−	−	−	+	+	+	+	+	+
B	1	+	+	+	+	+	+	−	−	−	−	−	−
	2	+	+	+	+	+	+	−	−	−	−	−	−
	3	+	+	+	+	+	+	−	−	−	−	−	−
	4	+	+	+	+	+	+	−	−	−	−	−	−
	5	+	+	+	+	+	+	−	−	−	−	−	−
	6	+	+	+	+	+	+	−	−	−	−	−	−

TABLE IV. Results obtained by pairing in all possible combinations twelve cultures from single conidia developed on normal X non-conidial hybrid.

basidiospores in precisely the same way as the single basidiosporal mycelia react. Furthermore both A and B conidia are produced on one conidiophore.

MUTATION AND HYBRIDIZATION

As mentioned earlier, one single conidial culture differed from all others in macroscopic and microscopic characters. This culture was obtained from the germination of a single conidium which had been produced on a normal diploid clamp-bearing

mycelium resulting from the mating of two normal haploid mycelia (basidiospores 1 and 12). Of the twenty-five single conidial cultures isolated, twenty-four resembled the parents in type of colony formed and in microscopic characters, while one culture was noticeably different. This culture grew much more rapidly than either of the parent cultures (FIG. 1*a*) and produced more abundant aerial mycelium, so that the colony appeared cottony and much whiter than the normal colony (compare FIG. 1*a* and 1*b*). Microscopic examination revealed an entire absence of conidiophores and the presence of many short curved branches which are probably responsible for the cottony appearance of the colony (FIG. 7). These characters have persisted through the three months since this culture was obtained, and have remained constant through several transfers of the mycelium.

It would seem, therefore, that this non-conidial form is to be interpreted as a mutant in the haploid, having originated as the result of some nuclear change which occurred during the formation of the conidium or in nuclear divisions immediately preceding conidial formation.

This non-conidial mutant (number 22 in Tables II and III) has the ability to form hybrids[2] when paired with suitable haploid mycelia. From Table II it is seen that it reacts in such a way as to form clamp-bearing mycelium with conidial cultures 2, 7, 9, 10, 23, 24, 25, 27 and 28. From Table III it is clear that the mutant 22 belongs to the same group (group B) as the parent basidiospore culture 12, and has the ability to form clamp-bearing mycelium when mated with basidiospore culture 1 (group A). Therefore the mutation has not affected the ability to react in such a way as to form clamp-bearing mycelium when mated with a haplont of opposite group.

The diploid clamp-bearing mycelium obtained by mating the

[2] The term "hybrid" is commonly used to describe the first diploid generation obtained by the pairing of gametes produced by two dissimilar diploid parents. In the present paper the term is used to refer to the thallus (dicaryophyte) obtained by mating two dissimilar haplonts. The nuclei in the cells of such a dicaryophyte remain separate until the fusion in the basidium. While the situation is obviously different than in truly diploid organisms, it has seemed best for the purposes of this discussion to use the term "hybrid" in this general sense, rather than to introduce a special terminology.

non-conidial mutant with a suitable haplont originating either from a basidiospore or a conidium, differs from the normal diploid mycelium resulting from the pairing of two normal haploid mycelia. Macroscopically the hybrid cross resembles the mutant parent in that it grows at approximately the same rate and develops considerable aerial mycelium, so that the resulting colony has an appearance similar to that of the mutant (FIG. 2*b*). Microscopic examination shows that the clamp-bearing hyphae exhibit the same type of branching as that of the mutant (FIG. 8), this apparently being associated with the cottony appearance of the colony. Unlike the mutant, however, the diploid colony produces conidiophores and conidia, in this respect resembling the normal parent, although the formation of conidiophores takes place at a somewhat later stage in the development of the colony and in less abundance than on the normal.

Such a hybrid cross was obtained by growing together non-conidial mutant 22 (group B) and single conidial culture 24 (group A), and the same technique as was described above was used to insure the purity of the diploid clamp-bearing mycelium. Conidia from this culture were sown on plates of cooled malt extract agar. While the colonies were still small (up to 3 mm. in diameter) they were examined, using the low power of the microscope. The two types of colony were readily distinguishable, the normal type being small and producing conidia while still minute in size, the mutant type being relatively larger and without conidia. In this way two hundred and ninety-five colonies were examined. Of this number, 142 were of the normal conidia-producing type like the parent culture 24, 153 were of the non-conidial type resembling the mutant parent 22.

Numerous attempts were made to germinate separately the conidia from an individual conidiophore, but many difficulties have been encountered. However, in one case two conidia from a single head germinated, and the two mycelia produced were isolated. One of these resembles the normal parent in growth characters and conidial production, the other the non-conidial parent. When paired, these two mycelia give rise to a typical hybrid cross colony. This, along with the evidence obtained by examination of many colonies resulting from conidia borne on

the hybrid cross, shows that both the original nuclei, one bearing the factor for conidial production, one for conidial sterility, were involved in the formation of conidia on a single head.

Twenty-four of these single conidial cultures were isolated, twelve of the normal conidial type, twelve of the non-conidial type. These have remained constant.

Six of the non-conidial haploid mycelia were paired in all possible combinations with six of the normal haploid mycelia, the results being tabulated in Table IV. It is evident that the non-conidial mycelia 1 to 6 fall into one group, conidial mycelia 18 to 23 into another group. An individual of the first group when paired with an individual of the second group is able to react in such a way as to form clamp-bearing mycelium. Since the non-conidial mycelia are all of the same reaction type it may be assumed that they are identical with non-conidial parent 22, and similarly that the conidial mycelia are identical with normal parent 24.

DISCUSSION

It has been established that in *Peniophora Allescheri* uninucleate conidia are produced on both haploid and diploid mycelia. The spores from the haploid serve to repeat that generation, those from the diploid re-establish haploid mycelia identical with the two types of haploid mycelia which entered into the formation of the diplont. Thus new haploid mycelia and new hybrids can be formed at all stages during vegetative activity. If mutations occur in nature as seems possible since a noticeable one has arisen during the short time this fungus has been under observation, then the mechanism exists for including such mutants into diploid hybrids immediately. Furthermore, the two types of haplonts entering into the diplont could be recovered through the haploid conidia produced on it. This would allow for repeated hybridizations and so make possible the establishment of many such hybrids in place of the original one.

It has been stated previously that the diploid clamp-bearing mycelium resulting from the pairing of the non-conidial mutant with a normal conidial haplont resembles the non-conidial parent in macroscopic appearance and excessive branching, and the

conidial form in production of conidia. Cytological work shows that such a clamp-bearing mycelium is binucleate and it is accepted that in each pair one nucleus is a descendant of a nucleus derived from one parent, the other a descendant of a nucleus derived from the other parent. It may be assumed that in such a cross the nucleus from the non-conidial parent is influencing the type of development of the diploid mycelium in such a way that it resembles that parent in rapidity of growth and excessive branching. On the other hand the nucleus from the normal conidia-bearing parent is influencing the type of development of the diploid mycelium so that it resembles the normal parent in the production of conidia. This, then, indicates clearly that the two nuclei of the pair, though existing independently in the cells, coöperate in determining the characters of the diploid colony. The effect would appear to be comparable to that normally expected in a first generation hybrid in plant and animal groups having diploid nuclei. The diploid thallus obtained by mating two different haplonts shows biparental characters.

Binucleate hybrids in rusts and smuts have been studied and there also, the two nuclei of the pair each share in determining the characters of the diplont. Using pathogenicity as a criterion Newton, Johnson and Brown (**8**) found that hybrids formed by crossing physiologic forms of *Puccinia graminis Tritici* were "intermediate in pathogenicity between the two parental forms" and Levine and Cotter (**5**), by crossing strains of *P. graminis Secalis* and *P. graminis Tritici*, obtained a strain of stem rust capable of attacking certain varieties of rye and wheat which neither of the parents alone could do. In comparable experiments in smuts Goldschmidt (**3**) hybridized two strains of *Ustilago violacea*, each of which was able to infect only one host, and produced a diplont capable of attacking both hosts. Using morphological characters as a criterion, Newton, Johnson and Brown (**9**) observed that in a cross of strains of *P. graminis Tritici* involving two spore color types, one being present in each parent, the resulting hybrid showed the presence of both characters.

In all these cases studies have been made on binucleate hybrids and it has been established that such hybrids combine the

characters of the parents. It may be concluded, therefore, that in crosses such as these and in *Peniophora Allescheri*, in which two nuclei are associated but not fused, both of the nuclei are effective in determining the nature of the resulting diplont.

Subsequent analyses of the progeny of such hybrids have been made by Zattler (11) on *Schizophyllum commune* Fries and *Collybia velutipes* Curt. and by Johnson, Newton and Brown (4) on *Puccinia graminis Tritici*. Up to the present time the basidiospores of *Peniophora Allescheri* have not been obtained in culture. Observation of the hymenium formed in nature shows characteristic fusion of nuclei in the basidia and subsequent production of four nuclei and four uninucleate spores. If formation of perfect fruiting bodies in the hybrid can be obtained in culture, analyses of the progeny will be undertaken.

It should be emphasized, in conclusion, that the study of the hybrid in *Peniophora Allescheri* has been confined to an analysis and comparison of the vegetative characters and conidial production of the two haplonts involved in the cross, with similar characters of the resulting hybrid dicaryophyte or diplont. In the papers cited above the comparison of characters is between two generations of the diplont, the characters of the intervening haplonts not having been considered. A heterothallic basidiomycete, such as *Peniophora Allescheri, in the vegetative phase*, may be considered as having an alternation of "homologous" haploid and diploid generations. Since these may be grown separately in pure culture it is possible to compare vegetative characters of the haploid thalli with corresponding characters of the diplont. Such a direct comparison would not appear to be possible in other groups of the fungi, though certain green, brown and red algae having an alternation of homologous generations would seem to offer similar opportunities.

ACKNOWLEDGMENT

This study has been carried on under the direction of Professor H. S. Jackson, to whom I wish to express my appreciation for his constant interest and stimulating suggestions. I am also indebted to Professor L. O. Overholts of Pennsylvania State College for the specific identification of the fungus and to Dr. G. W. MacArthur for reading the manuscript.

SUMMARY

1. *Peniophora Allescheri* Bres. is genetically heterothallic.

2. Oedocephaloid conidiophores are produced on haploid and diploid mycelia. Conidia are uninucleate in both cases, those on the haploid mycelium repeating the haploid generation, those on the diploid mycelium giving rise to haploid mycelia of the two parental types, as determined by ability to react to form clamp-bearing mycelium.

3. A mutant in the haploid has appeared which differs from the normal haploid mycelium in type of growth and in its inability to produce conidia.

4. Mating this non-conidial mutant with a suitable normal haplont produces a diploid colony which reveals characters of both parents and bears conidia which when grown separately give rise to colonies, in equal numbers, having the characteristics of the two parents.

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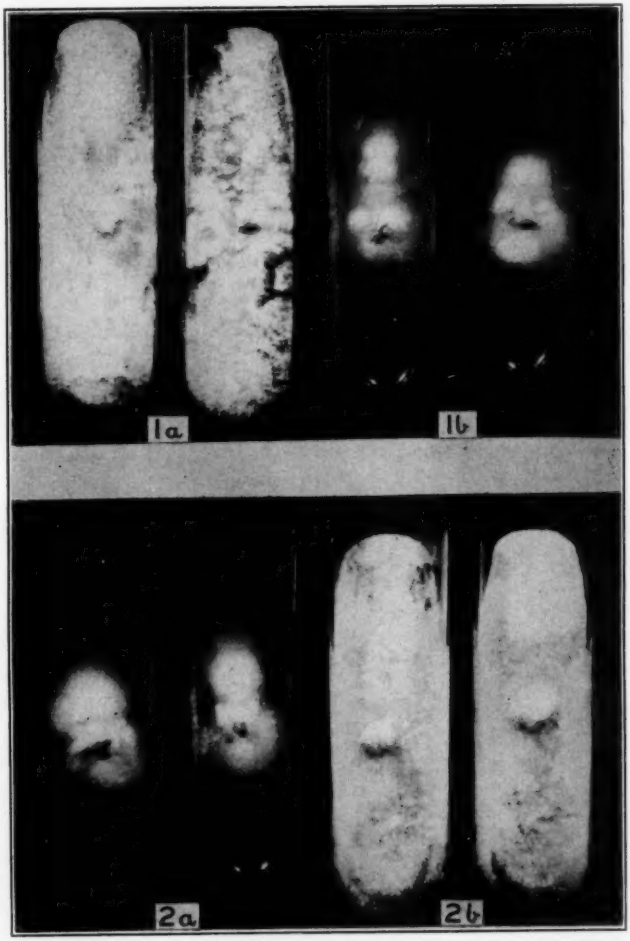

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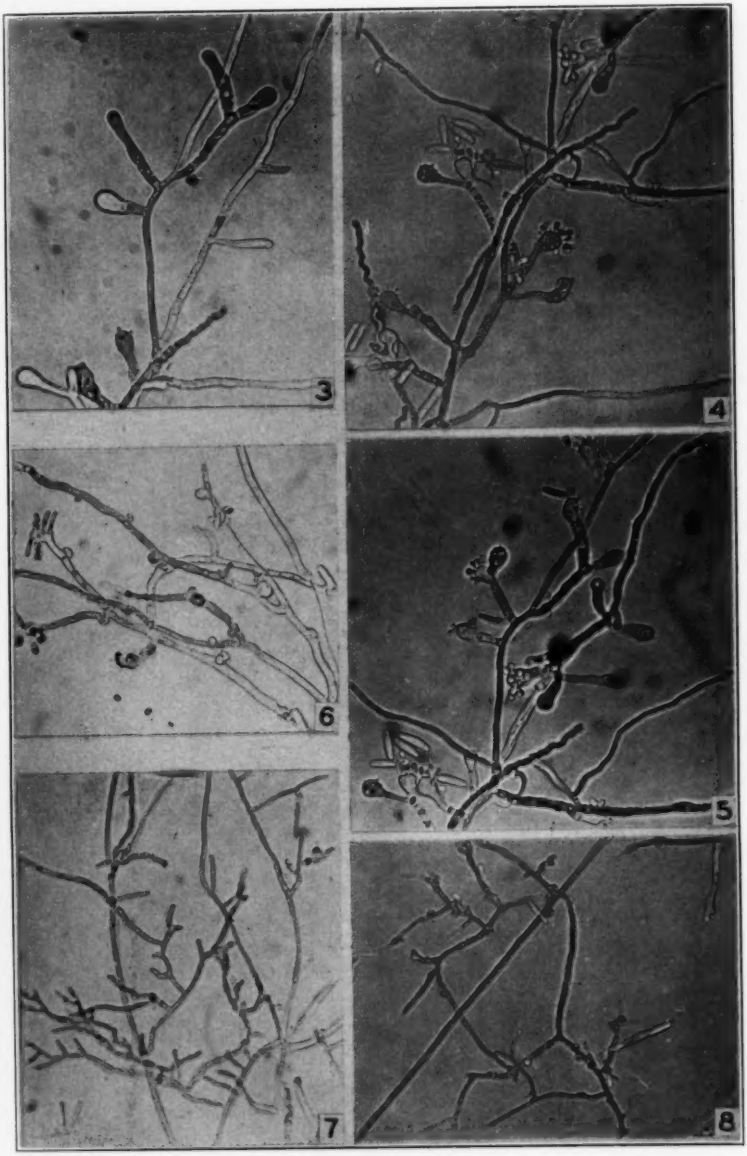

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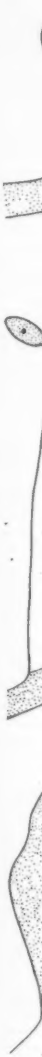

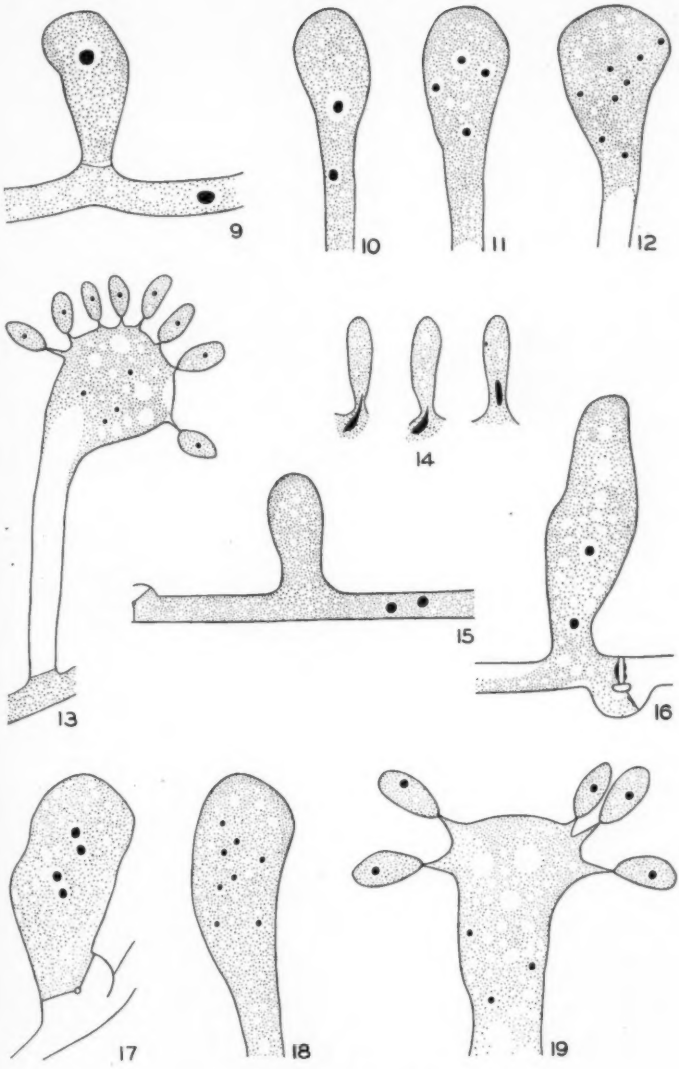

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EXPLANATION OF PLATES 21–23

Fig. 1*a*, cultures of haploid non-conidial mutant 18 days after inoculation; 1*b*, cultures of normal haploid of the same age; 2*a*, cultures of normal diploid obtained by pairing 2 suitable conidial haplonts; 2*b*, cultures of hybrid obtained by pairing a normal conidial haplont and the non-conidial mutant; 3, haploid mycelium and young conidiophores. × 360; 4, the same field as that in fig. 3 taken twenty-four hours later. × 360; 5, conidiophores and conidia on the haploid mycelium. × 360; 6, clamp-bearing mycelium and conidiophores. × 360; 7, mycelium of the non-conidial mutant. × 360; 8, mycelium of the hybrid obtained by pairing a normal haplont and the non-conidial mutant; 9–13, stages in conidiophore development on the haploid mycelium. × 2600; 14, nuclei migrating into developing conidia. × 2600; 15–19, stages in conidiophore development on the diploid mycelium. × 2600.

BROWN ROT OF FRUITS AND ASSOCIATED DISEASES IN AUSTRALIA

T. H. HARRISON

(WITH PLATE 24 AND 2 TEXT FIGURES)

PART II. AN INTERESTING DISCOMYCETE, *SCLEROTINIA AESTIVALIS* POLLOCK, OCCURRING ON MUMMIFIED FRUITS

INTRODUCTION

In the spring of 1921, the author found apothecia of *Sclerotinia fructicola* (Wint.) Rehm., arising from apricot mummies in an orchard at Pennant Hills, near Sydney, New South Wales, Australia (**3**). In December of that year, he revisited the orchard and found, on several mummied apricots, numbers of small, pinkish, discoid apothecia. Some thousands of similar apothecia were later collected from mummified apricots and quinces, while numbers were also obtained from mummified apples, plums, peaches and pears. The constant association of these apothecia with fruits mummified by the brown rot fungus, led to the assumption that perhaps they were the apothecia of that fungus, an assumption not verified by microscopic examination.

Many features of this fungus, however, make it one of great interest to those working with the brown rot fungi, and also to mycologists generally, particularly to those engaged in teaching work. Some of these features are: (1) It is very easily obtained in pure culture from ascospore clouds; (2) it appears to have been recorded only once previously—by Pollock in Michigan, U. S. A., in 1909 (**7**); (3) crop after crop of apothecia may be obtained from the one mummied fruit, and this may extend for a period of up to 13 years; (4) in pedigree cultures obtained from single ascospores, it will, on artificial media in the laboratory, reproduce apothecia in a few months; (5) it has not produced a macroconidial stage, but microconidia are at times abundant; (6) it is normally a saprophyte, growing vigorously upon an unusually

wide range of nutritive media; (7) it appears to be capable of replacing the brown rot fungus in mummied fruits.

HISTORICAL

In 1909, Professor J. B. Pollock (7) of Ann Arbor, Michigan, U. S. A., recorded, as follows, a new species of *Sclerotinia:*

Sclerotinia aestivalis Pollock n. sp. Since the preceding part of this paper went to the printer a new species of *Sclerotinia* has been collected at Palmyra and Ann Arbor, Mich., first by Dr. L. H. Pennington, June 26, 1909, then by myself and several of my students through July and up to Aug. 9. It occurs on old mummied apples that have lain on the ground over winter. It is apparently not a parasite on either the apple or plum, since ascospores inoculated into ripe and unripe apples both sound and bruised did not develop a *Monilia* stage, nor cause a rapid rot of the apples. Similar negative results were obtained on inoculating the ascospores into plums. After several weeks in the moist chamber, a very slow rot on both takes place.

Apothecia from one to fifty on a single mummied apple; stalks short, rarely reaching 1 cm., not more than .5 mm. thick, dark brown and tapering below, lighter colored above; disc from 1–7 mm. in diameter, light reddish brown, flat when mature, radiating ridges and furrows below, not hairy but appearing almost velvety on outside; asci somewhat clavate or nearly cylindrical, short for the genus 51–85 × 6–8.5 mostly 68–75 micromillimeters long, not turned blue by iodine, several from a common stalk when teased out; ascospores narrowly elliptical, 6.4–11.9 × 2–3.4, average size about 8.5 × 3 micromillimeters.

This species differs from the other species of the genus occurring on fruits in the time at which the apothecia develop. They are usually produced in April or May, while this one occurs in midsummer, on account of which the species name *aestivalis* is chosen for it.

The conidial stage was searched for where the ascus fruits were found, but none was found. Attempts were made to get cultures of the ascospores as they were shot into the air, but no conidial stage developed. Whether there is no conidial stage, or whether the right conditions for its development have not been offered, is not at present ascertained.

This record was unsupported by illustrations, but specimens of the fungus were preserved. Pollock did not publish further details of this fungus.

In 1912, Demaree (1), while working in Professor J. B. S. Norton's laboratory, described a *Sclerotinia* which he had found on apples at College Park, Maryland, U. S. A., in November, 1911. His description agreed fairly well with that of Pollock's *S. aestivalis*, but Demaree did not know of this fungus. He was unable to produce growth from ascospores although he tried various media including fruit and cooked apples. He contrasted his fungus with Aderhold & Ruhland's *S. fructigena*, and supposed

the former to be the perfect stage of a fungus causing brown rot of apples in America.

Specimens of Demaree's fungus have not been preserved at College Park, Maryland.

J. B. S. Norton *et alii* (**6**), in 1923, made the following statement: "Demaree, in 1912, described a *Sclerotinia* on Maryland apples, which may be *Sclerotinia aestivalis* Pollock." In 1932, Norton saw specimens of *S. aestivalis* Pollock, and again expressed the opinion that this was probably the fungus which Demaree had. In the absence of specimens and illustrations of Demaree's fungus, however, it is impossible to be sure of this point, particularly as it is difficult to understand how anyone, who had *S. aestivalis* Pollock, could fail to produce growth from ascospores.

In 1928, the author (**4,** p. 140) made passing reference to this fungus. He stated that mummied apples collected near Sydney, early in 1922, "were producing apothecia of *S. aestivalis*," and that on 19th September, 1922, apothecia found on mummified apples, "consisted of the small flattened apothecia of *S. aestivalis*," and of *S. fructicola*. An illustration of the two apothecia on a mummied apple was published.

OCCURRENCE IN AUSTRALIA

ON APRICOTS. On December 25, 1921, beneath the apricot tree, under which apothecia of *S. fructicola* had been obtained in the previous September, the author found three mummied apricots, bearing a number of very small reddish brown, shortly stipitate apothecia. On the following day, an intensive search was made beneath apricot trees in an adjoining orchard. Fruits mummified, in the previous season, by the brown rot fungus were very abundant, especially in shallow depressions caused by the cultivation methods adopted. Under twenty trees selected for search, from 1 to 30 per cent of the mummied fruits each bore from 2 to 30 apothecia. Most of these were produced from the point where the half-buried mummied fruits made contact with the ground surface, but some fruits, in the moister spots, bore apothecia from fully exposed surfaces. The diameter of the disc varied from 0.5–6 mm., while the length of stipe

varied from 0.5–8 mm.—depending largely upon location. In some cases germination of the seeds in the mummied fruits was in progress, and one seedling, with apothecia of this fungus on the sclerotioid mass surrounding the seed, is illustrated in plate 24, fig. 4. One typical apothecium was found arising from the petiole of an apricot leaf, which was lying amongst a number of mummied fruits, most of which bore apothecia (PLATE 24, FIG. 5). It is very probable that the leaf was originally killed by the brown rot fungus, because twig blight was prevalent in the orchard during the previous season.

ON QUINCES. In the same orchard there were several quince trees, beneath which large numbers of apothecia were found on mummied quinces. The abundance of the apothecia is indicated by the fact that one group of 17 mummied quinces, or parts thereof, bore a total of 816, *i.e.* an average of 48 per quince. One quince bore 170 apothecia, most of which were mature. Excellent clouds of ascospores were expelled when this and other specimens were moved. Since 1921, the author has collected apothecia of *S. aestivalis* on quince mummies on many occasions, whenever required for class purposes. The mummied fruits apparently become thoroughly absorbed by the *S. aestivalis* mycelium, because they have an astonishing ability to produce many crops of ascocarps over an extended period. Some of the quinces bearing *S. aestivalis*, collected in December, 1921, have since been kept in his laboratory by Dr. W. L. Waterhouse, University of Sydney. Each year, at intervals dictated by class requirements, he has moistened the mummied fruits, which are set in soil in a seed pan, and he has not yet failed to obtain a crop of apothecia. Some quince mummies bearing *S. aestivalis* were collected by the author in February, 1928, and used for the production of several crops of apothecia at Hawkesbury College. An illustration of one such crop is shown in plate 24, fig. 1. Some of these mummied quinces were dried and taken to England in June, 1930. After being in an envelope for four months, the mummied fruits were placed in moist, sterile sand, and incubated, for a few days, at 25° C. A heavy crop of apothecia was produced for exhibition at the November meeting of the British Mycological Association in 1930. Since that time several further

crops have been produced, as required, from the same mummied fruits.

Another feature of importance is the longevity of the individual apothecia, some of which have continued to produce spores over a period of 5 weeks, in most cases growing appreciably meanwhile. Further, while many apothecia are mature, others are just commencing to emerge from the substratum. Individual mummied fruits have yielded apothecia continuously over a period of 4 months.

TIME OF DEVELOPMENT

The species name, *aestivalis*, was chosen by Pollock because he found the apothecia in midsummer, in contrast to the spring apothecial development of the Brown Rot fungus, *S. fructicola* (Wint.) Rehm. In New South Wales, the apothecia occur in greatest numbers in the field from December to March, but have also been noted in April, August, and September. In the laboratory at Sydney University, apothecia were developed continuously from December, 1921 to February, 1923. It would appear, however, that relatively warm temperatures are necessary for the development of the apothecia, and these are normally found at soil level, in weedy orchards, during midsummer in New South Wales. The optimum temperature for vegetative growth has been shown by experiment to be approximately 25° C., and this temperature has been used successfully to initiate apothecial development.

SIZE OF APOTHECIA

The size of the apothecia found on quinces varied greatly. It appeared to be partly dependent on the number of apothecia present on a mummied fruit. In one case where three apothecia occurred on a mummied fruit, the average diameter of the discs was 5.43 mm., while of 68 measured on a single fruit, the average diameter was 2.56 mm. The average diameter of the discs of 457 apothecia, collected in December, 1921, was 2.7 mm., but the range extended from 0.25 mm. to 10.5 mm. The size, however, of any individual apothecium is not constant, shrinkage taking place in a dry atmosphere, swelling in a moist one. Moreover, an apothecium may be mature and eject spores, when its diam-

eter is only 0.5 mm., and provided that moisture and suitable temperature are available, it may continue to grow to a diameter of 5–8 mm. It is clear, therefore, that the diameter of the disc in this fungus is so very variable that, alone, it is of little value for taxonomic purposes.

ON PLUM. On 14th January, 1922, following climatic conditions similar to those prevailing three weeks previously, 4 mummied plums, bearing apothecia, were found beneath a Japanese plum tree, in an orchard at Pennant Hills. One mummied plum bore 6, another 40, while the other two had intermediate numbers of apothecia present. The apothecia were mostly small, the largest having a diameter of 2 mm., while the longest stipe was also 2 mm. Conditions at this time were not very favourable for apothecial growth, although suitable for initiating their production.

ON PEAR. In April, 1921, Dr. W. L. Waterhouse placed a Keiffer's Hybrid pear, which had been rotted and mummified by the brown rot fungus, in his garden for observation. In February, 1922, he found a single apothecium of *S. aestivalis* arising from this mummied pear, which was brought to the University. After being placed on moist soil, and incubated for a period, this mummied pear gave a number of apothecia of this fungus, but none of *S. fructicola*.

ON APPLE. On 8th March, 1922, beneath a thick carpet of weeds such as *Fumaria officinalis* L., and *Plantago lanceolata* L., surrounding an apple tree in a moist part of an orchard at Pennant Hills, large numbers of apothecia of *S. aestivalis* were found. In some cases they arose from almost entire mummied apples, but in others they came from pieces of skin-like material, which ranged from the merest fragments to pieces representing half or more of the apple. In April, 1922, in another orchard in the same district, apothecia were found on mummied apples of the Trivett Seedling variety. In September of the same year, more apothecia were developed, in seed pans at the University, from mummified fruits collected in April (**4,** p. 140).

ON PEACH. In March, 1922, mummied peaches were collected at Pennant Hills, taken to the University, and there placed in soil. From one of these an apothecium, of the normal *S. aestivalis* type, was subsequently developed.

DETAILS OF APOTHECIA

Colour. The colour varies considerably with the age of the apothecia, and with the amount of moisture present. Normally, however, the typical colour may be described as flesh pink, darkening appreciably to reddish-brown, and later to brown when drying and turning mealy with age. In terms of Ridgway's Standards (**8**), the range is from pale flesh pink to cornelian red, with the majority falling within the colour range between flesh colour and carrot red.

Size. As detailed in connection with quinces and apricots, the size of the disc is most variable. Pollock records that the diameter varied from 1 to 7 mm., while the author, with more material available, found the range greater, recording disc diameters ranging from 0.25 to 10.5 mm.

Shape. The first signs of the apothecia are small, rounded, pink coloured protuberances on the surface of the mummied fruit. These are gradually lifted above the surface by elongation of the stipes. The end of the stipe is flattened soon after emergence, but quickly becomes concave, to form the apothecium. At first crateriform, the apothecium becomes campanulate, often flattened and, in some cases, convex. The margin is obtuse, glabrous, and usually remains entire.

Stipe. The apothecia are positively phototropic, hence the length of the stipes is governed largely by the point of origin of the apothecia. Of 105 apothecia arising from mummied quinces, the average stipe length was 3.445 mm., but in this set the range was from 0.25 to 12.5 mm. The greatest stipe length noted by the author was 27 mm.

Usually the stipe is unbranched, but several examples of two and three apothecia on a branching stipe have been noticed (PLATE 24, FIG. 6). On several occasions, apothecia have been the point of origin of further apothecia—the stipes arising from the centre of the old discs. One such interesting specimen had a branched stipe bearing two apothecia. From each of these apothecia, three further apothecia were produced, making eight in all from a single point of origin. Another unbranched stipe bore a medium sized apothecium, from which seven stipes were sent up to bear small apothecia.

The colour of the stipe ranges from the black of the substratum in which it originates to the pink colour of the disc. The stipe is usually thin, gradually expanding to form the base of the disc. Rhizoids are absent.

Nature of Substratum. A point of considerable taxonomic significance in the *Sclerotinieae* is the nature of the material from which the apothecia originate. In this case, the apothecia arise from fruits mummied by the brown rot fungus, *i.e.* from a structure which, consisting of fungus and incorporated host tissue, has been defined by Honey (**5**) as a pseudosclerotium. This pseudoclerotium, however, is all host tissue for the fungus, *S. aestivalis*. No sclerotia were found in association with the apothecia, either on the mummied fruits or on the apricot petiole. In pure culture, however, flattened sclerotioid bodies, from which apothecia develop, are produced in the medium.

The Asci. The shape of the asci is indicated in text figure *A*. The size ranged from 56.6–81.7 $\mu \times$ 4.8–7.7 μ for 200 asci measured. Size depends considerably upon the time, in development, at which the measurements are made. In this case, asci showing mature spores in a distichous arrangement were measured.

Spores are arranged at first in a monostichous manner, but towards maturity they move towards the top end of the ascus, overlapping one another and eventually becoming distichous just prior to expulsion. Spores are ejected forcibly from the ascus when the pressure of the surrounding atmosphere is reduced, producing a conspicuous cloud, which has been photographed.

The top end of the ascus is rounded until the ascospores assume the distichous arrangement, when, just prior to ejection of spores, it becomes flattened. When the spores have been ejected, the end is square and somewhat frayed. The pore stains blue with iodine.

The hymenium contains asci at all stages of development at any one period (TEXT FIG. *A*, FIG. 5). This probably explains the unusually extended period during which ascospores may be ejected from a given apothecium.

Paraphyses vary in size, being sometimes shorter but usually slightly longer than the asci. They are usually 2–3-septate, hyaline, filiform, single or branched, only slightly swollen at the apex.

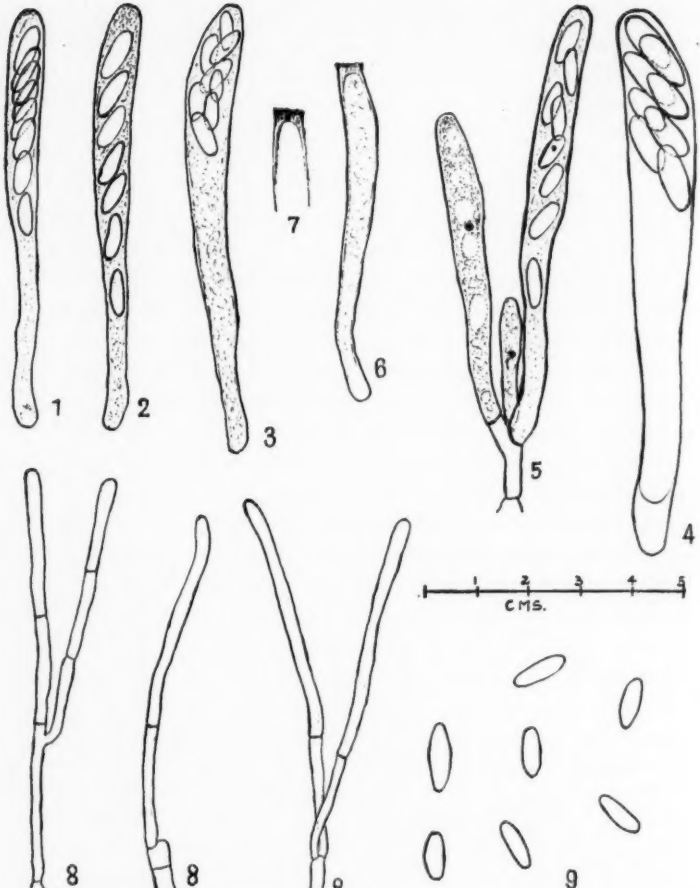

FIG. A. 1–4, asci showing various arrangements of ascospores; 1 subseriate, 2 monostichous, 3 and 4 distichous; 5, portion of hymenium, showing asci in various stages of development; 6, ascus from which spores have been ejected. Note frayed square end; 7, ascus as in 6, stained with iodine, showing central dark blue pipe, indicating the position of the pore; 8, paraphyses—single and branched; 9, a group of ascospores. All drawn with the aid of a camera lucida. Magnification, in original plate, 1000 except 4 which is × 1400. The reduction for this text figure is indicated by the centimetre scale included.

Ascospores. The spores are hyaline, elongated, ellipsoidal, with sharply rounded ends (TEXT FIG. *A*, FIG. 9). They have no conspicuous oil spots or vacuoles. The spores measured 6–10 $\mu \times$ 2–3.5 μ, with an average of 8.56 $\times$ 2.97 μ for 300 spores measured.

Germination of the ascospores takes place rapidly in distilled water, or upon the surface of many nutritive media. At 25° C., in 2 per cent dextrose or in prune juice decoction, it is very rapid. Germination from both ends of the spore is usual, although one germ tube is generally somewhat earlier than the other. The germ tube is surrounded by a gelatinous sheath, easily shown in Indian ink or gentian violet. Branching occurs freely, and, while not regular, is always lateral, and usually at an angle greater than 45° (TEXT FIG. *B*).

Septa soon appear and the cells vary in size. Some hyphae are constricted at the septa, others not. Adjacent cells in a hypha may show the two conditions, while the diameter of the hyphae is also most variable. It appears, therefore, that these features of the germ tube and vegetative hyphae have no taxonomic significance for this fungus.

Method of Obtaining Single Spore Cultures. Ascospores, ejected by mature apothecia, were collected on clear 2 per cent plain agar, in inverted petri dishes. The dish was held in such a way that a well distributed seeding of ascospores was obtained on the surface of the cooled agar. The closed petri dish was left at laboratory temperature for a period of 14–24 hours, dependent on temperature. The closed petri dish was inverted on the stage of the microscope, and with the low power objective (16 mm.), the surface of the agar was located and searched for sporelings. The position of well isolated sporelings was marked by a drop of Indian ink, the centre of which was immediately removed by a small pointed piece of blotting or filter paper. This left the sporelings immediately above the centre of a ring of Indian ink. When as many sporelings as desired were located in this way, the petri dish lid was removed and with a special platinum needle, the agar, containing the desired sporelings, was cut away from the surrounding agar, but not removed at this stage. The ink markings were then removed from the bottom of the petri dish,

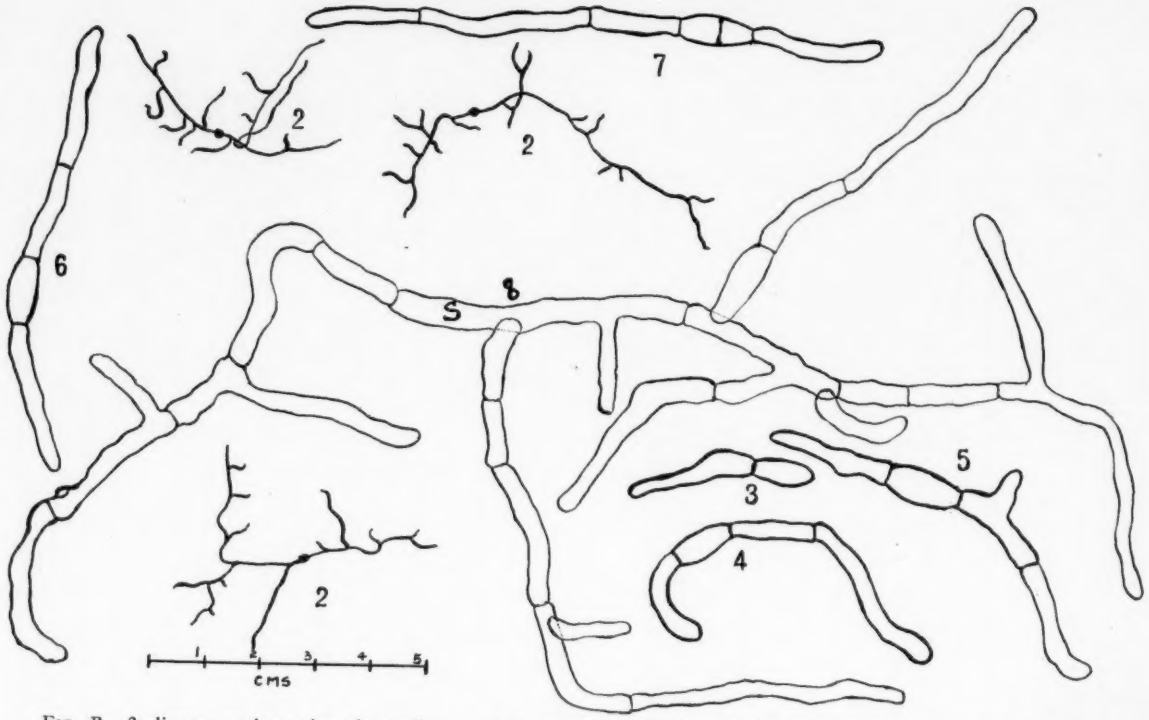

FIG. *B*. 2, diagrammatic tracing of sporelings on dextrose 2%, the position of the spore being indicated by the dots; 3–7. germination, in prune juice decoction, at room temperature at Sydney, January, 1922. All drawn 20–22 hours after planting; 8, sporeling on 2% dextrose agar, held for 24 hours at 25° C. and for 18 hours at 15° C. All drawn with the aid of a camera lucida. The magnification for the original plate is 1000 except No. 2 where it is × 200. The variation from this is indicated by the scale incorporated in the plate.

and the fact that the sporelings were uncontaminated, and completely isolated in an island of agar, was verified by microscopic observation. With a 10 × eyepiece, a 16 mm. objective was usually sufficient, but, if in doubt, an objective such as 4 mm. was used. The isolated sporelings were then removed on their platforms of agar to the edge of nutrient slopes, and again the purity of the culture was verified by microscopic observation. The growth was then watched carefully, so that no chance of contamination occurred.

GROWTH ON ARTIFICAL MEDIA

Sclerotinia aestivalis has been grown under observation upon the following media:

Nutrient agar in tubes and petri dishes: potato dextrose, maize meal, prune juice 2 per cent, malt extract, mummy extract, oatmeal, Brown's a, Coon's, Richard's and 2 per cent plain agar; *sterilised plugs* or *portions* of the following: potato, prune, pear, apple, French bean pod, radish, parsnip, carrot; *stems* of tomato, castor oil plant, maize, lucerne; *petioles* of celery, cowpeas, silver beet, rhubarb; *crushed grains* of maize, wheat, barley, oats, rye, rice, soybeans; *leaves and bark* of the Turpentine (*Tristania laurifolia*); and *shoots* of eschalot.

In all cases portions of the above were placed in tubes, and a few ccs. of distilled water added. They were sterilised in the autoclave at 15 lb. pressure for 20 minutes. After inoculation, the tubes were incubated at 25° C., and detailed observations made at intervals.

It is a tribute to the tolerance of the fungus to changes in the nutritive medium, that it was able to grow on all the above materials. In all cases there was produced a surface and subaerial mycelial growth, white and flocculent at times, but devoid of a macroconidial stage. Usually there was formed beneath the surface mycelium, a dark brown to black sclerotial crust, which may extend to cover the exposed area of the medium. Considerable nigrescence of the medium, to varying depths, takes place.

Macroconidial Stage. The author has tried many times to induce production of an imperfect spore stage, but, so far, all efforts have failed. This fungus has not only been grown on a

The magnification for the original plate is 1000 except No. 2 where it is × 200. The variation from this is indicated by the scale incorporated in the plate.

wide range of media, but has been subjected to rapid changes in environment, and also to starvation methods, which have proved successful in inducing conidial development in other fungi. Every likely culture has been examined microscopically. In some preparations, hyphae have been noted with very numerous septa, giving the appearance of chains of immature chlamydospores, but none of these has been observed to develop further, and to abstrict.

It is possible that this fungus is devoid of a macroconidial stage—a condition in agreement with Whetzel's contention that the fungus is a *Ciboria*.

Microconidia, agreeing in shape, size and method of production, with those described for other members of the *Sclerotinieae*, have been observed.

Return of Apothecia in Pure Culture. In May, 1922, a monospore isolate, obtained from an apothecium on quince, was subcultured on malt extract agar in a petri dish. This culture was kept at room temperature, in a laboratory at the Agriculture School, Sydney University. On August 16, *i.e.* approximately 15 weeks later, one of the sclerotioid bodies, which had been formed in the medium, bore a mature apothecium. Further apothecia were later produced from this and other sclerotioid bodies (PLATE 24, FIG. 7).

In December, 1930, the author supplied Mr. F. T. Brooks of Cambridge with a single ascospore culture, isolated in London. Brooks subsequently reported that in a large petri dish, 4 months after subculturing onto P.D.A., he obtained a large crop of apothecia. The apothecia in this case, too, arose from thick, black, flattened, sclerotioid bodies, developed at intervals in the medium.

A monospore culture of *S. aestivalis* was also supplied to Professor H. H. Whetzel, at Cornell, who obtained abundant crops of apothecia in artificial media.

While it is possible that the monospore culture, from which apothecia arose in Sydney in 1922, may have become "contaminated" with another "strain" of *S. aestivalis*, it is impossible in the two other cases. A monospore culture, supplied by the author in each case, was the only material of *S. aestivalis* avail-

able. This evidence shows clearly that *S. aestivalis* is homothallic.

REACTION ON FRUITS

In February, 1923, selected Trivett seedling apples were surface sterilised with alcohol, and inoculated with an active culture of *S. aestivalis*, in comparison with the *S. fructicola* (Wint.) Rehm., and *S. sclerotiorum* (Lib.) De Bary. Detailed observations were made over a period of 5 weeks, the apples being kept under bell jars in the laboratory. It was shown that at the end of the period, *S. aestivalis* had extended only through an area of 6 mm. on either side of the wedge of apple, cut out for inoculation purposes.

The observation was verified by a separate experiment in 1923 and on several occasions in London in 1931. *S. aestivalis* can produce a very slow dark brown rot in apples, but is not capable of causing complete rotting and mummification of fruits. It could not have been responsible, therefore, for the mummification of the fruits on which it was found in such abundance.

Further work is contemplated on the relationship of *S. fructicola* and *S. aestivalis*, in view of the almost constant association of the latter with fruits mummified by the former. That the association is actual may be illustrated by the following example. In 1930, Dr. W. L. Waterhouse, of Sydney, forwarded to London 13 quinces which he knew to be mummified by the brown rot fungus. From 8 of them, in England, the author developed apothecia of *S. aestivalis*.

Contrast with S. sclerotiorum (Lib.) De Bary. It has been suggested that the fungus described above was merely *S. sclerotiorum* (Lib.) De Bary. On many occasions, the author has isolated this fungus from infected plant tissue in Australia, and has grown it in culture on a large number of media, as well as on various fruits. He has also developed the apothecia to maturity in pure culture. Not only is this fungus strikingly different in cultural behaviour and parasitic ability, but morphological features of taxonomic significance are different from those in *S. aestivalis*, *e.g.* the asci of *S. sclerotiorum* are twice as long as those of *S. aestivalis*. The author also had *S. aestivalis* growing under comparable conditions with many strains of *S.*

sclerotiorum in England during 1931. He also saw much of Ghamrawi's comparative work upon *S. sclerotiorum* and *S. Trifoliorum* Erik. The author is convinced that *S. aestivalis* Pollock is a species distinct from both *S. sclerotiorum* (Lib.) De Bary and *S. Trifoliorum* Erik.

THE TAXONOMIC POSITION

Through the courtesy of Dr. C. L. Shear, of the U. S. D. A., some of the type material, supplied by Professor J. B. Pollock, has been studied. In all morphological details, this material agreed with that in the possession of the author, and with Pollock's description of it.

A study of the foregoing pages will show that the author, by virtue of the larger quantity of material available, has found a greater range in certain features than is quoted by Pollock, but in essential morphologic details, Pollock's description remains an excellent one of the fungus found in Australia. The Australian fungus is, therefore, cospecific with that described by Pollock in 1909.

NOMENCLATURE

The historical review shows clearly that the only name proposed for this fungus is *Sclerotinia aestivalis* Pollock. It is properly published, with an adequate description, and, therefore, the specific name *aestivalis* must stand irrespective of the suggestion to follow.

The close affinities existing between *Sclerotinia*, *Ciboria*, and others of the family *Helotiaceae* are generally recognised, but Fuckel's original generic criteria have proven unsatisfactory. Professor Whetzel has found it necessary to revise and define more clearly the generic concepts in the sub-family *Sclerotinieae*. In March, 1932, Whetzel, after a study of the author's material of *S. aestivalis*, expressed the view that the fungus should be included in the genus *Ciboria*, rather than in *Sclerotinia*, because the sclerotioid bodies were produced *in* the medium and not free from it, as is the case with a typical *Sclerotinia*. It has been stressed above that the apothecia in nature arise directly from the host tissue.

In August, 1932, monospore cultures of this fungus were forwarded to Whetzel, who, on December 15, 1932, wrote to the author as follows: "I am pleased to report that S 961, (a monospore culture of *S. aestivalis*) produced apothecia in abundance for me. It is clearly a *Ciboria*, and a most typical one. In your future publications on this form you need have no hesitation in placing it in the genus *Ciboria*."

The author feels justified in accepting Whetzel's authority for the change of the generic name.

The new combination, *Ciboria aestivalis* (Pollock) Whetzel, is proposed for the fungus described in this paper.

SUMMARY

1. An interesting Discomycete associated with fruits mummified by the brown rot fungus, *S. fructicola* (Wint.) Rehm., is recorded for the first time in Australia.

2. Taxonomic details are presented to show that it agrees with a fungus described as *Sclerotinia aestivalis* by Pollock in Michigan, U. S. A., in 1909.

3. In New South Wales, it has been found on mummied apples, apricots, peaches, pears, plums and quinces. Details of its occurrence on each host are given.

4. The apothecia are very abundant. On a quince mummy, 170 apothecia were present at the one period. Many crops are produced from the one mummied quince. Some have given apothecia each year for 13 years.

5. The method adopted by the author for isolating pedigree cultures from single ascospores is described. The fungus grows readily on an extensive range of artificial media.

6. No macroconidial stage has been found, despite careful searches on many media, kept under varying conditions calculated to induce sporulation.

7. Apothecia have been obtained in pedigree cultures on artificial media, held in laboratories in Australia, England and America, for 3–4 months.

8. For practical purposes it may be regarded as a saprophyte, although it will cause a very slow rotting of apples, when held for long periods under favourable conditions for the growth of

the fungus. It may be parasitic upon the pseudosclerotium of the brown rot fungus, *S. fructicola* (Wint.) Rehm.

9. On the authority of Professor H. H. Whetzel of Cornell University the fungus is transferred from the genus *Sclerotinia* to *Ciboria*.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge indebtedness to Dr. W. L. Waterhouse for inspiration and guidance during the early part of the work and for the photographs; to Dr. C. L. Shear for securing the type material; and to Professor H. H. Whetzel for helpful suggestions.

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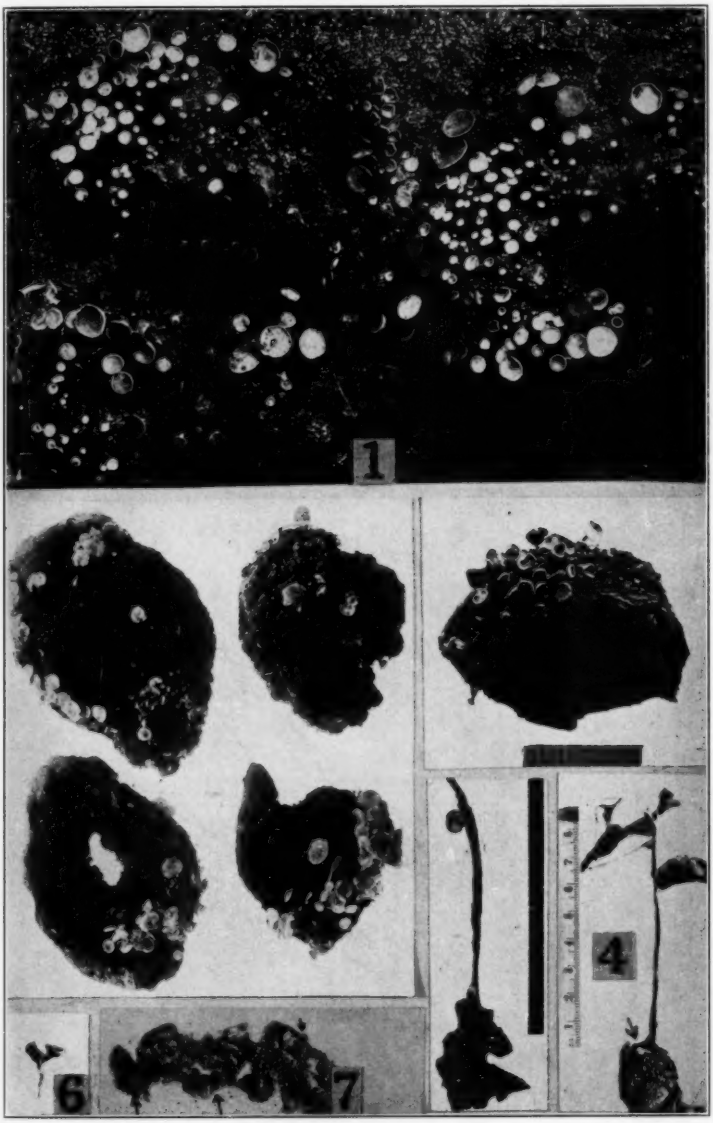
EXPLANATION OF PLATE 24

Sclerotinia aestivalis Pollock. Fig. 1, crop of apothecia produced at Hawkesbury College, N. S. W., from mummied quinces collected at Pennant Hills, N. S. W., in February, 1928. × 1.25; 2, apothecia from mummied quinces, one of which bore 170 specimens. × 0.92; 3, cluster of apothecia on mummied apricot. × 1.25; 4, apricot seedling arising from seed enclosed by a black pseudosclerotium bearing apothecia of *S. aestivalis*. × 0.36; 5, apothecium arising from the petiole of an apricot leaf. × 1.5; 6, three apothecia arising from a single stipe. × 1.25; 7, apothecia on petri dish culture of M. E. A. Note the dark sclerotioid bodies which were produced in the medium. Natural size. All material illustrated in fig. 2–6 was collected at Pennant Hills, N. S. W., in December, 1921. Photographs by Dr. W. L. Waterhouse.



SCLEROTINIA AESTIVALIS

EXPERIMENTS WITH HETEROECIOUS RUSTS[1]

GEORGE B. CUMMINS

AECIDIUM HYDNOIDEUM BERK. & CURT.

Field observations made by the writer near Lafayette, Ind., in 1931 indicated that this conspicuous rust on *Dirca palustris* might form its alternate stage on *Carex*. June 3, 1932 the aecia were collected and the spores used to inoculate *Carex pennsylvanica*. A scattered production of uredia was evident June 18; no telia developed. Attempts to infect the *Dirca* with teliospores found associated with it in the field have been unsuccessful, the plants never remaining alive after removal from the field to the greenhouse.

The urediospores obtained from this culture agreed with those of *Puccinia extensicola* and formed the basis on which Arthur in the "Manual of the Rusts in United States and Canada," page 200, created the variety *P. extensicola hydnoidea*.

PUCCINIA CARICIS-STRICTAE DIET.

Through the kindness of Mr. Roy Latham of Orient, Long Island, N. Y., the writer was supplied with *Carex stricta* collected January 31, 1933 and bearing amphispores and teliospores of *P. Caricis-strictae*. The teliospores germinated freely in April and were used on April 3, 1933 to inoculate young plants of *Urtica dioica*. An abundant infection developed with pycnia appearing April 9 followed by aecia on April 17. The same telial material was again used on April 12, 1933 and gave equally positive results on 12 pots of *Urtica dioica*, the pycnia appearing April 17 and the aecia April 25.

A comparison of this rust with the common *Urtica-Carex* rust, *Puccinia Caricis*, proved the two to be similar. Arthur (*l.c.* p. 208) used this culture as the basis for his erection of the variety *P. Caricis Caricis-strictae*.

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

This culture is especially interesting in that it indicates that so-called amphisporic species may be only variants of rusts not producing amphispores in the normal life-cycle. If this is true it can be predicted that *P. atrofusca* will produce aecia on species of *Artemisia*, the chief aecial hosts of *P. universalis* (*Dicaeoma Dracunculi*). These two forms were united by Arthur (*l.c.* p. 205) under the name *P. atrofusca*. In like manner the aecia of *P. vexans* may be expected to occur on the Asclepiadaceae and to agree with those of *P. Bartholomaei*. In attempts to culture *P. vexans* I have never been successful in germinating the few teliospores which have been found. This rust ordinarily is found only in the amphisporic condition.

PUCCINIA SMILACIS SCHW. AND AECIDIUM APOCYNI SCHW.

This species has long been considered to be autoecious but field observations communicated by Mr. Roy Latham indicated that this was not true. Although uredia and telia commonly occurred on Long Island he never observed the development of aecia on the same host, *Smilax rotundifolia*.

Teliospores on *S. rotundifolia* collected at Mattituck, L. I., N. Y., by Mr. Latham were used in an unsuccessful attempt, made June 16, 1933, to infect plants of the same species at Lafayette, Ind. Mr. Latham then suggested that *Aecidium Apocyni* might belong to the *Smilax*-rust. He kindly furnished old aecia of *A. Apocyni* collected at Mattituck in association with *P. Smilacis*, August 3, 1933. No germination was secured on slides and no infection resulted from an inoculation of *Smilax rotundifolia*.

Mr. Latham again collected telia on *S. rotundifolia* at Orient, Long Island, December 26, 1933 and kindly sent me a supply which were over-wintered at Lafayette. Inoculation of plants of *Apocynum cannabinum*, also furnished by Mr. Latham, May 15, 1934 resulted in an abundant infection, the pycnia appearing May 23 followed by aecia on and after June 3.

The only known collections of *Aecidium Smilacis* were made in North and South Carolina. This aecial form can no longer be considered to belong to *P. Smilacis*. That *A. Smilacis* may belong to *Puccinia Arundinariae* was suggested by Arthur (*l.c.*

p. 383). Through the kindness of Mr. M. A. Rice of Clemson College, S. C., I have been able to over-winter teliospores of the *Arundinaria*-rust at Lafayette but have not been able to germinate the spores and cannot, therefore, report on the correctness of the above suggestion.

PURDUE UNIVERSITY,
LAFAYETTE, INDIANA

NOTES AND BRIEF ARTICLES

Buller's Researches on Fungi

Volume VI of the above work has recently appeared and exhibits the same painstaking care and patience which have characterized previous volumes.

Part I is devoted to the *Pilobolus* gun and its projectile. It is here shown that *Pilobolus Kleinii* and *P. longipes* can both shoot their sporangia vertically to a height exceeding six feet or a horizontal distance of eight feet. The mechanism of this discharge is fully discussed. The sporangium has the form of a plano-convex lens and in landing nearly always does so with the flat gelatinous side next to the object on which it lands. The sporangia thus tightly adhering to blades of grass have often been mistaken by mycologists (including the writer) for fruiting bodies of parasitic fungi. The sporangia thus placed are readily devoured by herbivorous animals and on release germinate and grow. Chapter three of part I is devoted to *Pilobolus umbonatus*, described as a new species on page 178 of this volume.

Part II consists of a description of the phenomenon of puffing in *Sarcoscypha proteana* and other discomycetes. In many of the cup-fungi the spores are shot to a distance of several centimeters above the hymenium and are then apparently dependent upon currents of air for their further distribution. The puffing is often accompanied by an audible sound as has been frequently noted.

Part III consists of a description of *Omphalia flavida* and *Sclerotium coffeicola*, two fungi parasitic on the leaves of coffee. The entire volume consists of 513 pages and 231 illustrations. Like previous volumes it is published by Longmans, Green and Company. F. J. Seaver

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The Recovery of a Pathogenic Fungus from Leucocytes

During an investigation conducted to determine the cause of a nasal granuloma [1] in cattle in Louisiana a fungus was isolated

[1] Nasal granuloma in cattle in Louisiana. N. Am. Vet. **15**: No. 9. 1934.

which grew readily and fruited abundantly on Sabouraud medium at blood heat or more slowly at ordinary room temperature.

Guinea-pigs were inoculated with cultures of this organism, and after 7–10 days were found to have developed mycosis at the point of inoculation, in the omentum and the liver. Leucocytes from these lesions were found to contain 1–3 or more irregularly spherical, refractive bodies which in culture medium sent out a germ tube which became septate and branched. From these structures fruiting cultures were developed in 48 hours.

A paper on the morphology and systematic position of this fungus is in preparation.

VERA K. CHARLES

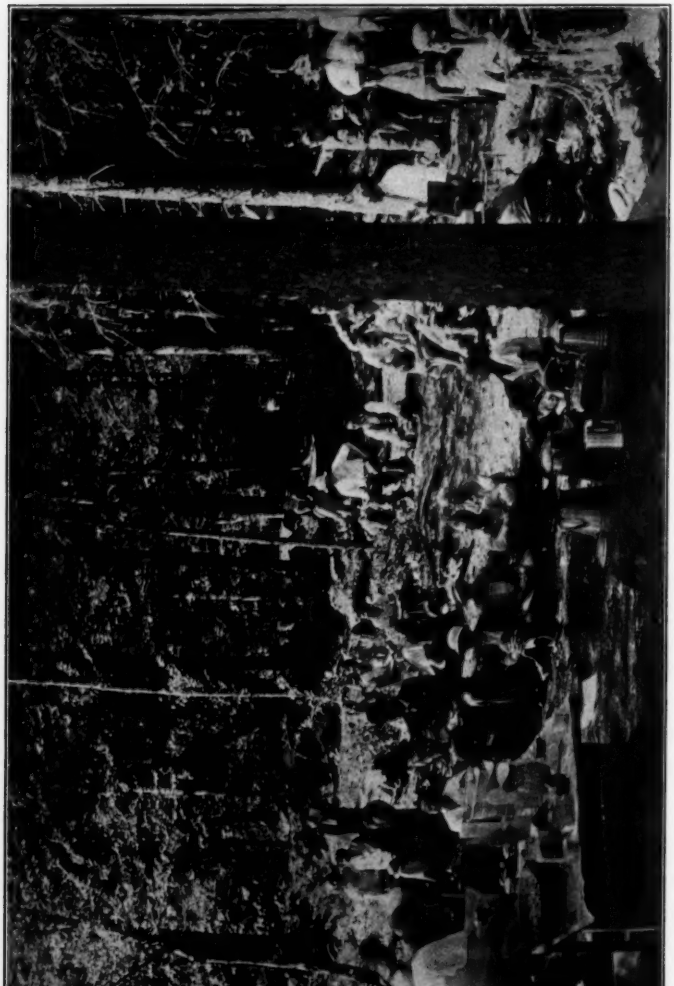
BUREAU OF PLANT INDUSTRY

The Mycological Society of America

SUMMER FORAY

The second annual Foray of the Mycological Society of America was held at Seventh Lake, near Inlet, New York, August 21st–24th. The meeting was well attended by about forty-five mycologists and pathologists, twenty-seven of whom were members of the Mycological Society. Four of the present officers and members of the council were present, and among others such well known figures as Drs. R. A. Harper, C. L. Shear, John Dearness and C. Thom. Non-mycological members of families and guests brought the total attendance to seventy.

The Headquarters for the Foray was at the summer camp of Dr. F. C. Stewart on the north shore of Seventh Lake. Some of the visitors were accommodated at the camp, the majority, however, in summer hotels in the vicinity. The boat house at the camp was fitted for a laboratory, including microscopes and a good working library—part of which was loaned from the Department of Plant Pathology at Cornell and part from the Geneva Experiment Station. Facilities for drying mushrooms and pressed material were provided in a tent nearby. Another laboratory was provided at Johnston's camp near Sixth Lake, where facilities for handling parasitic fungi were provided under the leadership of Professors Whetzel and Welch of Cornell



Summer foray; picnic on Seventh Lake.

University. Lunch and dinner were provided each day for the entire party by Mrs. Stewart and daughters in an eminently successful manner.

Collecting on the part of those interested in the Basidiomycetes was done mostly in the immediate vicinity of Seventh Lake under the leadership of Dr. Stewart. Those interested in the parasitic fungi, under the leadership of Professor H. H. Whetzel, visited several outlying localities, thus dividing the party on several occasions into two groups referred to as the "fleshies" and the "parasites." On Friday, following a trip to Bug Lake by the "fleshies" and to Blue Mountain by the "parasites," the two groups came together at noon at a camp site between Seventh and Eighth Lakes for a most excellent picnic lunch provided by Mrs. Stewart and helpers. Following this lunch the party was entertained most successfully by Mr. Hoffmaster with a clever sleight of hand performance.

While the collecting could be considered only fair on account of the rather dry weather preceding the meeting, an abundance of material was obtained in all groups. Perhaps the most notable collection on which a report is available at this writing was that made by Mr. Robert Hagelstein, honorary curator for Myxomycetes at the New York Botanical Garden, who obtained 76 species and 3 varieties of Myxomycetes. From several dung collections made at the time of the Foray, Dr. R. F. Cain, of the University of Toronto, has since obtained the development in moist chamber cultures of 29 species of "Sordariaceae" including 8 European species previously recognized in North America only in Ontario, and 7 of the species described as new in his recent paper on this group. Mr. J. L. Lowe collected a large number of Lichens but no report is yet available. Drs. L. O. Overholts, A. H. Smith and W. H. Snell among others made extensive collections of the higher Basidiomycetes. A partial list including some of the less common fungi which were collected is given below with the name of the person or laboratory sponsoring the report.

The unqualified success of the Foray was due in no small measure to the untiring efforts of Dr. Stewart, who, with the able assistance of our Secretary-Treasurer, Dr. H. M. Fitzpatrick and others from the Cornell Laboratory, spared no effort to

Summer foray; picnic on Seventh Lake.

make the occasion one to be long remembered by those who were fortunate enough to be able to attend. Special mention is due Mrs. Stewart, who was responsible for the most excellent meals.

NOTEWORTHY COLLECTIONS

Ascomycetes: *Adelopus balsamicola* (Peck) Thiess., on *Abies balsamea*, coll. Cornell; *Bifusella Faullii* Darker, on *Abies balsamea*, coll. Darker; *Diaporthe Pruni* Ellis & Ev., coll. Wehmeyer; *Diaporthe Macounii* Ellis & Ev., coll. Wehmeyer; *Enchnoa lanata* (Fries) Sacc., coll. Wehmeyer; *Geoglossum alveolatum* Durand, coll. Jackson; *Gloeoglossum affine* Durand, coll. Jackson; *Hypoderma rufilabrum* (Berk. & Curt.) Duby., on *Acer spicatum*, coll. Jackson & Darker; *Hypodermella nervata* Darker, on *Abies balsamea*, coll. Darker; *Lasiosphaeria muscicola* DeNot., coll. Cornell; *Lophodermium filiforme* Darker, on *Picea rubra*, coll. Darker; *Mollisia Iridis* (Rehm) Sacc., coll. Cornell; *Pezicula minuta* Peck, on *Viburnum*, coll. Wehmeyer; *Trichoglossum Walteri* (Berk.) Durand, coll. Jackson; *Ascocalyx Abietis* Naumov., on *Abies balsamea*, coll. Jackson; *Lachnum myricaceum* (Peck) Sacc. (*Peziza myricacea* Peck) on *Myrica gale*, coll. Shear; *Bolina atrovirens* Ellis & Ev., on *Fagus*, coll. Shear.

Thelephoraceae: *Aleurodiscus Farlowii* Burt, on *Abies balsamea*, coll. Jackson; *Corticium botryoideum* Overh., coll. Overholts; *Peniophora viticola* (Schw.) H. & L., coll. Overholts.

Clavariaceae: *Clavaria subfalcata* Atk., coll. Jackson; *Physalacria inflata* (Schw.) Peck, coll. Overholts.

Polyporaceae & Boletaceae: *Boletus placidus* Bon., coll. Snell; *Boletus leucophaeus* Pers., coll. Snell; *Boletinus spectabilis* Peck, coll. Snell; *Polyporus semisupinus* Berk. & Curt., coll. Snell; *Polyporus benzoinus* (Wahl) Fries, coll. Snell; *Trametes mollis* (Sommerf.) Fries, coll. Overholts.

Agaricaceae: *Crepidotus stipitatus* Kauff., coll. Smith; *Hypholoma hololanigerum* Atk., coll. Smith; *Lacterius aspideoides* Burl., coll. Smith & Snell; *Mycena margaritispora* Lange, coll. Smith; *Mycena subviscida* Kauff. & Sm., coll. Smith; *Pholiota intermedia* Smith, coll. Smith; *Pluteus coccineus* (Mass.) Beard., coll. Smith.

Uredinales: *Calyptospora goeppertiana* Kuhn, III on *Vaccinium pennsylvanicum*, coll. Cornell; *Chrysomyxa Cassandrae*

(Peck & Curt.) Tranz., I on *Picea mariana*, coll. Cornell; *Glomerularia Lonicerae* (Peck) Dearness & House, on *Lonicera canadensis*, coll. Cornell; *Milesia fructuosa* Faull, I on *Abies balsamea*, coll. Darker; *Pucciniastrum Arcticum* (Lagerh.) Tranz., II on *Rubus triflorus*, coll. Cornell; *Pucciniastrum Potentillae* Kom., II on *Potentilla tridentata*, coll. Cornell.

Fungi Imperfecti: *Cercospora Callae* (Peck) Clinton, coll. Cornell; *Cercospora gentianicola* Ellis & Ev., coll. Cornell; *Coccospora aurantiaca* Wallr., coll. Cornell; *Gelatinosporium fulvum* Peck, coll. Wehmeyer; *Fusicladium radiosum* (Lib.) Lindr., coll. Cornell; *Rhinotrichum repens* Preuss., coll. Linder; *Septoria Scutellariae* Thüm., coll. Cornell; *Septoria Wilsonii* Clinton, coll. Cornell; *Dendrodochium epistroma* v. Höhn. on *Diatrypella betulina* (Peck) Sacc., coll. Shear; *Macrophoma parca* (Berk. & Br.) Berl. & Vogl. on *Abies balsamea*, coll. Shear.

H. S. JACKSON

ANNOUNCEMENT FOR 1935 SUMMER FORAY

The Council of the Society at the Atlantic City meeting delegated to the Vice-President the function of selecting the time and place for the summer foray. In accordance with this procedure Vice-President Dearness has announced that the 1935 foray will be held at Ithaca, New York, August 20–23 inclusive. The mycological laboratories of the Department of Plant Pathology in the Plant Science Building of Cornell University will serve as headquarters. The local committee in charge of arrangements will consist of Professor H. H. Whetzel and the Secretary-Treasurer. The Ithaca members of the Society extend a cordial invitation to all mycologists to attend.

Lodging and meals can be obtained at reasonable rates at rooming houses and restaurants on or bordering the campus. All who plan to attend the foray are asked to advise the Secretary-Treasurer well in advance in order that adequate arrangements may be made. It is especially urged that the type of accommodations desired be indicated. Also it is important that those who intend to arrive by train shall so state, in order that automobiles may be obtained for their use while at the foray. Camp sites are available near by for any who desire to use tents.

Ithaca is located in the scenic Finger Lakes region of central New York at the head of Cayuga Lake, and is well known to botanists for the natural beauty of its surroundings, and the richness and variety of its flora. Within easy reach are fresh water marshes and lakes, peat and marl bogs, numerous small gorges with many waterfalls, upland woods, and open fields. High hills afford a variation in elevation of more than fifteen hundred feet with a corresponding difference in flora. In most seasons fungi occur in profusion, and in only the most unusual August is the collecting unsatisfactory. Pleasant days and cool nights are to be expected. The laboratories are well equipped with facilities for handling the materials collected. The herbarium, containing the specimens of Atkinson and his students is available for consultation.

Members of the Society and other students of the fungi are urged to make careful note of the dates of the foray, and to arrange their summer plans to include it. The success of the meeting last summer at Seventh Lake indicates a growing interest in this phase of the Society's activities.—H. M. FITZPATRICK, Secretary-Treasurer.

A committee named by the President at the Pittsburgh meeting submits the following resolution for publication in Mycologia:

During the year 1934, the Mycological Society of America has lost four of its members through death. The Society, therefore, expresses its deepest regret at their removal and extends its sympathy to the bereaved families. Those in whose memory this action is taken are: Charles E. Fairman; Mrs. Esther Lewis; Thomas H. Macbride; Frank Lincoln Stevens.

TWO NEW SPECIES OF NEUROSPORA

In the course of the writer's interspecific hybridization studies in *Neurospora* on which a report will be published soon, it was found that an eight-spored strain of *Neurospora*, collected and isolated in 1927 at Nanking, China, is distinct morphologically and physiologically from the other two eight-spored species of *Neurospora* so far described. Morphologically it is intermediate

between them, having an ascospore of the *N. sitophila* type, and a perithecium and ascus of the *N. crassa* type. It was formerly thought to be simply a hybrid of *N. sitophila* and *N. crassa*. But recent physiological studies by the writer proved the incorrectness of his early assumption, as the Chinese strain of the sex-reaction "a" produces on dextrose agar an abundance of saffron-colored conidia, quite distinct from the salmon-pink colored conidia of *N. sitophila* and *N. crassa* on the same medium. This Chinese strain also hybridizes rather readily with all of the species of *Neurospora* so far available and it is the only species that has to date been crossed with the new species from Porto Rico. It is, therefore, deemed proper to describe the Chinese strain as a new species.

Neurospora intermedia sp. nov.

Peritheciis gregariis vel sparsis, atro-brunneis, 310–640 μ in diametro, ostiolo papillari; ascis cylindricis, brevissime stipitatis, 170–220 $\mu \times 15$ μ, 8-sporis; sporidiis uniseriatis, ellipticis, viridi-atris, longitrorsum striatis, 19–26 $\mu \times 12$–15 μ, typice 23 $\mu \times 13$ μ.

Conidia in mass saffron-colored on dextrose agar, cantenulate, globose to ovate, 11–21 by 10–11 μ.

Hab. on corn cob, Nanking, China. Type deposited at The New York Botanical Garden and also at the Institute of Agricultural Research, Tsing Hua University, Peiping, China.

A four-spored species of *Neurospora* was collected by Dr. R. A. Toro in Puerto Rico, and the cultures were kindly provided for study by Dr. B. O. Dodge. This species does not hybridize, at least readily, with *N. sitophila*, *N. crassa* and *N. tetrasperma* as was found by Dodge [1] and recently confirmed by the writer. It is, however, usually fertile with the Chinese species, *N. intermedia*, described above. Again the component unisexual strains of the Toro species are fluffy when grown on dextrose agar, producing quite an abundance of yellowish conidia, while bright orange-colored conidia are produced by the strain of sex-reaction "a" of *N. tetrasperma*.

Morphologically it also differs from *N. tetrasperma* by its larger perithecium and also longer and narrower asci. The

[1] Dodge, B. O. The non-sexual and the sexual functions of microconidia of *Neurospora*. Bull. Torrey Bot. Club **59**: 347–360. 1932.

ascospores of the Toro species, although having the same range of size as those of *N. tetrasperma*, are as a rule longer and narrower. The ascus of *N. tetrasperma* according to the writer's measurement, is 128–195 μ in length and 19–24 μ in width, mostly 144 by 21 μ. Most of the ascospores are about 30 μ long and 16 μ wide. The size of the perithecium, varying much, does not seem to be a good criterion for distinguishing species of *Neurospora*. It seems that when few perithecia are formed, they are larger. Thus, the perithecium of *N. tetrasperma* usually has a diameter of 249 μ to 332 μ, but the diameter sometimes reaches 664 μ when few perithecia are produced.

Since the Toro strain is distinct morphologically and physiologically from the other four-spored forms of *Neurospora*, it is described as a new species.

Neurospora Toroi sp. nov.

Peritheciis gregariis vel sparsis, fuscis vel atris, 370–580 μ in diametro, ostiolo papillari; ascis cylindricis, stipitatis, 168–216 μ × 18–21 μ, typice 192 × 18 μ, 4-sporis, interdum 3 vel 5 sporis; sporidiis uniseriatis, oblongo-ellipticis, longitrorsum striatis, fuscis vel atris, 27–37 μ × 14–18 μ, typice 32 × 14 μ.[1]

Conidia pink-colored in mass on corn meal agar, yellowish on dextrose agar, conidia cantenulate, globose to ovate 8–16 by 8–11 μ.

Hab. in coffee soil in Puerto Rico. Type deposited at The New York Botanical Garden.

F. L. TAI

THE NEW YORK BOTANICAL GARDEN

A MUCOR FOUND IN FOWL[2]

While conducting nutrition experiments with young chickens, there was evident, a lack of thrift and a mortality not attributable to the type of feeding. Some of the dead birds and others that were killed were examined anatomically. In all cases the gizzard was highly inflamed and the mucous membrane had become separated from the heavy epithelial layer. The infected material

[1] Only the measurements of the ascospores in four-spored asci are given here.

[2] Journal Series paper of the N. J. Agricultural Experiment Station, Department of Seed Analysis.

from between the mucous and muscular coats was cultured upon nutrient agar, resulting in a pure culture of a filamentous fungus from all birds so examined.

When grown on nutrient agar, there were found monilioid hyphae and aerial budding similar to that of the *Monilia sitophila* group, but differing from the *Monilia albicans* type in which the yeast-like growth is predominant. This suggested a monilioid phase of a *Mucor*. Transfers made to sterile bread produced luxuriant growth and sporulation of a *Mucor* which belongs near to *Mucor hiemalis*, Wehmer (**1**) and *Mucor javanicus* Wehmer (**2**) and is probably a transitional form between the sub-genera, *Mono-* and *Cymo-Mucor* having both simple and sympodially branched sporangiophores. The monilioid phase of these two organisms has been cited by Lendner (**3**) as the Chinese yeast in oriental fermentation processes.

Whether or not this organism is the primary cause of the pathological condition or only a secondary factor is uncertain, for time and space to conduct confirmatory experiments were not available at the time. For this reason the information is reported as an observation.

This organism so obtained from the gizzards of chicks is added to Dr. Lockwood's collection of Mucorales under the name of *Mucor javanicus* 461

1. **Wehmer, C.** Der Mucor der Hanfroette, *M. hiemalis*, nov. spec. Ann. Myc. **1**: 37–41. 1903.
2. ——. Der javanische Ragi und seine Pilze, Zentralb. Bakt. Abt. II, **6**: 610–619. 1900.
3. **Lendner, A.** Les Mucorinees de la Suisse, Berne, 180 pages. 1908.

NANDOR PORGES, JULIUS F. MULLER, AND LEWIS B. LOCKWOOD
NEW JERSEY AGR. EXP. STATION,
U. S. DEP. OF AGRIC.

THE LICHEN FLORA OF THE UNITED STATES

The writer is especially interested in seeing this volume since he was closely associated with Dr. Fink, both in Iowa and since coming to The New York Botanical Garden. Miss Joyce Hedrick (Mrs. Volney H. Jones) is to be congratulated on her persistence in completing the work cut short by Dr. Fink's death.

As is well known, Dr. Fink believed that the lichens or algicolous fungi should be combined with parallel groups of non-algicolous fungi. The present volume has not gone as far in that direction as had been anticipated.

We note that the order Hysteriales is used as a lichen order, but includes only families of algicolous fungi, while Dr. Fink in previous publications included also the non-algicolous families, Hysteriaceae and Hypodermataceae. While this has not been done in the present volume, we assume that it was intended, otherwise the author would scarcely have been justified in using an ordinal name previously applied only to non-algicolous fungi. Undoubtedly other families of non-algicolous fungi will eventually be included also in the Lecanorales. However, the problem of deciding how non-algicolous fungi and the algicolous forms should be interspersed is a large one and Miss Hedrick apparently preferred not to deal with that problem here.

As indicated, most of the lichens of North America are ascolichenes, and it is a great convenience to have the species brought together under one cover. The volume consists of 426 pages of text, including an index of 33 pages, with all the species arranged alphabetically and the genus in parenthesis following, thus making it easy to locate any given species in the least possible time. The volume is illustrated with 47 plates consisting mostly of halftones with a few etchings. There are also a number of text figures taken from Schneider to illustrate the morphological characters of the lichen.

The introductory matter has been partly reprinted from the "Lichens of Minnesota," but modified somewhat to suit Fink's later views. One hundred and seventy-eight genera are treated in this volume. The specific diagnoses are brief and concise, an effort having been made to avoid repetition of generic characters. While the writer is not thoroughly familiar with lichen fungi, the book appears to be a very concise and thorough treatment of this group for the United States.

The volume is published by the University of Michigan Press, Ann Arbor, Michigan (price $4.00).—FRED J. SEAVER